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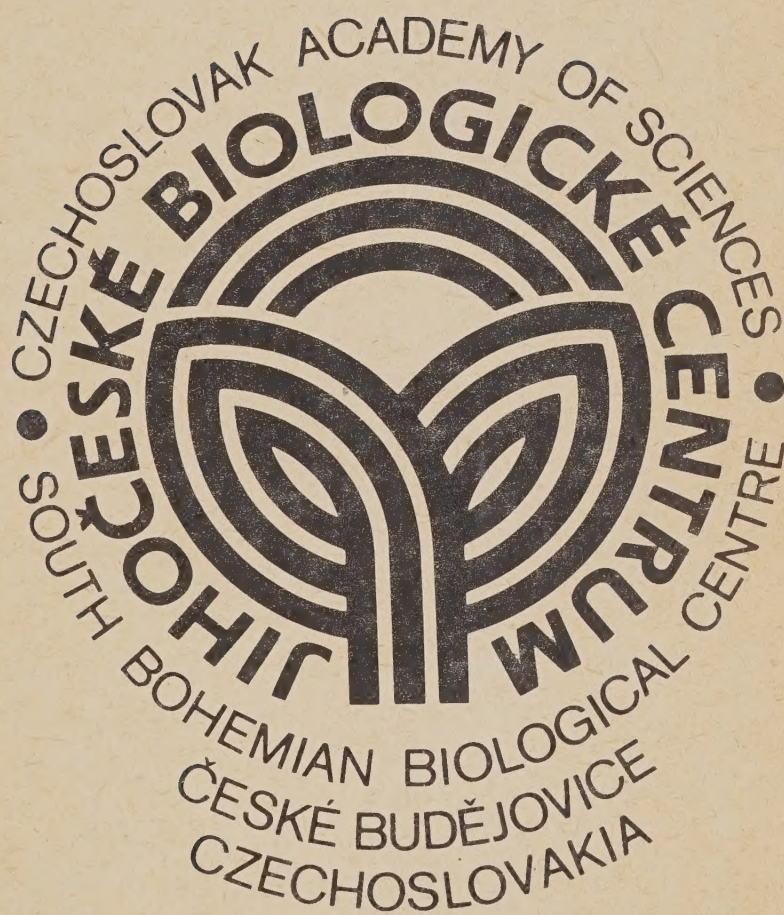
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BIOINDICATOIRES DETERIORISATIONIS REGIONIS

ČESKÉ BUDĚJOVICE, MAY 23-27, 1988

EDITED BY J. BOHÁČ AND V. RŮŽIČKA

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The emission indicators of microbiological pollutants from sewage treatment plants

A. KULIG

I N T R O D U C T I O N

Microorganism, like viruses, bacteria, and fungi are strictly connected with the sewage treatment process, because a big quantity of them participate in the reactions of biochemical decomposition of organic substances. Some of the microbes are, during the technological process, released from sewage to the atmosphere in the form of bioaerosol. Therefore, such objects as sewage treatment plants aerated sewage ponds, sludge drying beds, liquid waste removing fields, and sewage-sprinkled fields are artificial sources of microbiological pollutants emission (DART, STRETTON, 1977; OSSOWSKA-CYPRYK, 1987; RANDALL, LEDBETTER, 1966; WANNER, 1979).

Determination of kind and quantity of microorganisms in sewage is not a complicated task in the aspect of a method. Direct determination of momentary or a mean value of these microorganisms emission to the atmosphere is, however, difficult, since the emission takes place most often from surfaces within the range of several to several thousand square meters, and is unstable in the time, what means, it has a non-organized nature. Quantity of material airborne along with microbes depends upon size of the object, its technological parameters, meteorological conditions, and many other factors. Quantity of drops carried from above sewage treatment plant depends on arrangement of their diameters, what is influenced partly by working parameters of the diffuser, aerator or sprinkler, as well as by wind velocity, and condition of thermodynamic stability of the atmosphere. For example, CANNON (1983) stated on the ground of experiments, that aeration of sewage with a surface type mechanical instruments evoked higher emission of bioaerosol, than in case of aeration of sewage with compressed air. Microbial pollutants emission depends as well upon the concentration of microbes in sewage.

Majority of research on the influence of sewage treatment plant on microbiological quality of air refer to range of bioaerosol emission (KING, MILL, LAVRENCE, 1973; NEAPOLITANO, ROWE, 1966; OSSOWSKA-CYPRYK, 1982, 1984). These works do not, however, answer directly the question of quantity of pollutants emitted. Very few, as for example KENLINE et SCARPINO (1972) carried out research aiming at the determination of the kind and quantity of bacteria emitted to the atmospheric air. In this case, emission of bacteria found on agar from sewage treatment plant of hydraulic load being about 50.000 m^3 sewage per 24 h oscillates within the limits of $50 - 5000 \text{ bacteria m}^{-2}\text{s}^{-1}$ and the mean value was $440 \text{ bacteria m}^{-2}\text{s}^{-1}$.

Knowledge of quantity of pollutants emitted is necessary for comprehensive estimation of the influence of newlydesigned sewage treatment plants, and already existing ones upon quality of the atmospheric air.

METHODS

Research on microbial pollution was carried out with a measuring-calculating method (KULIG, 1986) rests on the use of direct measuring of the atmospheric conditions around the examined object, and on dependence tying quality of air with the emission and parameters influencing spreading of microbes. Scheme of the method is presented on Fig. 1. Values of microbiological pollutants emission were determined with the use of a Pasquill's, diffusion model (PASQUILL, SMITH, 1983). An example of application of this model to determine concentrations of microbes, coming from point emitter, is presented in the work by LIGHTHART et FRISCH (1976).

In the evaluation of emission there were also considered such parameters, as: shape and area of the surface, effective height of emission source, aerodynamic roughness of the surface of ground, velocity and direction of wind, thermodynamic stability of the atmosphere, and the microbial death rate. A process of sedimentation of aerosol particles was taken into account too, and pollutants reflection from the ground surface in dependence on wind velocity. The Appendix presents a detailed description of equations used to calculate emission.

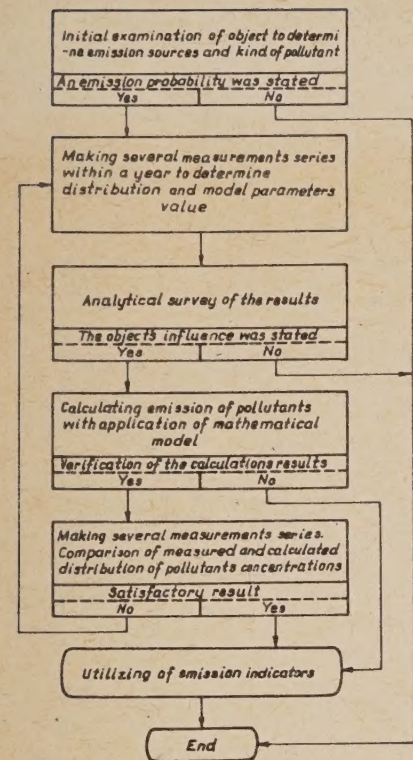


Fig. 1. Scheme of Measured - Calculated Method of determining quantity of microbiological pollutants emission from surface sources.

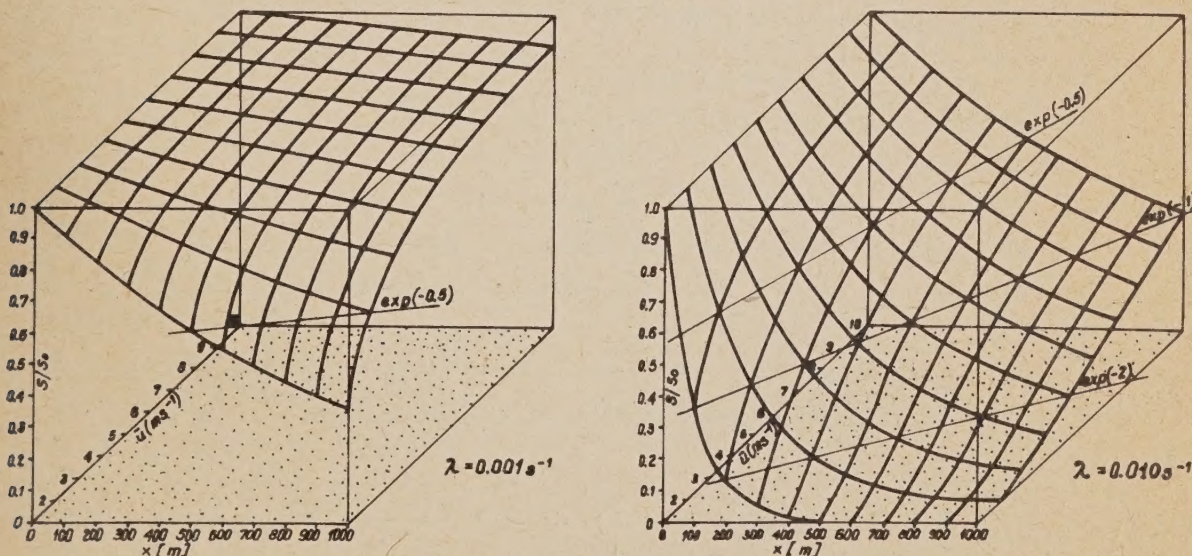


Fig. 2. Ratio S/S_0 versus downwind distance x from the source in meters and the mean wind velocity u in meters per second. S and S_0 are the downwind concentration of viable bacteria per cubic meter respectively with and without a microbial death rate λ per second.

Microbial death rate during the microbes' spreading in the atmosphere is a function of numerous factors, such as kind and physiological properties of the cells, relative humidity and temperature of air, oxygen concentration, intensity of visible, and ultraviolet radiation, and occurrence of air pollution. It is characterized by the microbial death rate λ the value of which may be determined through experiments. Exemplary values of the λ rate are presented in the work by LIGHTHART et FRISCH (1976), TELTSCH, SCHUVAL et TADMOR (1980). Influence of the microbial death rate on the value of the microbes concentration is expressed as follows:

$$s = s_0 \exp (-\lambda \cdot t)$$

where: S - concentration of microorganisms per cubic meter with the microbial death constant,
 S_0 - concentration of microorganisms per cubic meter without the microbial death constant,
 λ - microbial death rate per second,
 t - average time in second for transit of the microorganisms.

The dependence is presented on Fig. 2. It results, that, even at a small distance, decrease of microorganisms concentration because of their limited survival capacity, is significant and should be taken into account in any calculation of their spreading.

For the calculations there have been assumed values of microbial death rate equalling to 0., 0.001 and 0.01 s^{-1} . For the output values of factor, there were made in each case test calculations, and on the base of conformity of measurements with the calculations results, there was an ultimate factor value assumed.

Value of concentrations of microorganisms applied to evaluate emission were determined with the sedimentation method on the Petri dish covered with a proper nutrient medium. The result of the investigations carried out in the surroundings of the sewage treatment plant, is, that accuracy of the sedimentation method is about 20 % in relation to total viable particles (TVP).

RESULTS AND DISCUSSION

The investigations were conducted on five objects:

- Object A - the municipal sewage treatment plant "Bioblok" type WS-400; hydraulic load 400 cu. m /day,
- Object B - the dairy sewage treatment plant; hydraulic load about 800 cu. m/day,
- Object C - the yeast sewage treatment plant, hydraulic load about 1000 cu. m/day,
- Object D - the urban sewage treatment plant; hydraulic load about 100 000 cu. m/day,
- Object E - the sludge lagoons of municipal sewage treatment plant.

The sources with surface of emission smaller than 2500 m^2 (objects A, B, C) were replaced by a point emitter. The bigger sources (objects D and E) were replaced by a set of point emitters.

Object A

The main source of microorganisms emission is sewage aerating chamber equipped with surface aerators. The investigations were carried out within a year, and included 11 measurement series. The calculations were made of the following parameters: $h = 1$ m - total height of source, $P = 72$ sq. m, $z = 0.5$ m - height of measurement point, $z_0 = 0.01$ - for winter -

short grass 0.1 m, $z_0 = 0.06$ m - for summer - medium height grass 0.5 m, $u_p = 0.003$ m s⁻¹, $\lambda = 0$ s⁻¹ for TVP, $\lambda = 0.001$ s⁻¹ - for bacteria of the genera: Staphylococcus and Pseudomonas, $\lambda = 0.01$ s⁻¹ - for bacteria of the family Enterobacteriaceae. The following values of emission indicators for particular groups of microbes were obtained:

Total viable particles	59 040	microorg. m ⁻² s ⁻¹
Bacteria Enterobacteriaceae	15 200	microorg. m ⁻² s ⁻¹
Bacteria <u>Pseudomonas</u>	3 910	microorg. m ⁻² s ⁻¹
Bacteria <u>Staphylococcus</u>	3 210	microorg. m ⁻² s ⁻¹

In case of TVP it was stated, that emission value grew as the atmospheric unstability increased (PASQUILL, SMITH, 1983). The values may be represented in figures as follows:

moderately unstable conditions - class B	- 144 460 microorg. m ⁻² s ⁻¹
slightly unstable conditions - class C	- 34 790 microorg. m ⁻² s ⁻¹
neutral conditions - class D	- 16 440 microorg. m ⁻² s ⁻¹

The above dependence does not apply to other groups of microorganisms. Emission of microbial pollutants occurring in winter is significantly smaller than in the summertime. It is caused by technical operations, since in low temperatures period, aerating chambers are protected with special covers. Average values of emission indicators for the winter and summer are as follows:

	winter	summer
total viable particles	19 790	106 140 microorg. m ⁻² s ⁻¹
Bacteria Enterobacteriaceae	7 200	24 820 microorg. m ⁻² s ⁻¹
Bacteria <u>Pseudomonas</u>	4 930	2 300 microorg. m ⁻² s ⁻¹
Bacteria <u>Staphylococcus</u>	2 000	4 420 microorg. m ⁻² s ⁻¹

Object B

Main sources of the microbiological emission are: aeration chamber with a surface aerator, oxidation ditch with 12 aerating brushes, and sludge regeneration chamber. The grounds of the sewage treatment plant, from which an intense emission takes place, were assumed to be a surface source. Emission calculations were made for 21 series of measurements, assuming the following values of the model's parameters:

$h = 1$ m, $P = 1750$ sq. m., $Z = 0.5$ m, $z_0 = 0.06$ m,
 $u_p = 0.003$ ms⁻¹, $\lambda = 0$ s⁻¹ - for TVP, $\lambda = 0.001$ s⁻¹ -

for bacteria of the genera Streptococcus and Lactobacillus.

The following values of the emission indicators were obtained:

total viable particles	15 430 microorg. m ⁻² s ⁻¹
Bacteria <u>Streptococcus</u> and <u>Lactobacillus</u>	1 210 microorg. m ⁻² s ⁻¹

During research on object B there occurred slight changes in the atmosphere stability. Mean values of the emission were therefore, determined for classes B and C. They are as follows:

	class B	class C
total viable particles	20 210	12 710 microorg. m ⁻² s ⁻¹
Bacteria <u>Streptococcus</u> and <u>Lactobacillus</u>	1 400	1 150 microorg. m ⁻² s ⁻¹

Object C

The sewage treatment plant is composed of a series of process machines, however, the research refers merely to the oxidation ditch, which was treated to be the main isolated source of the emission of microorganisms. The ditch is point-aerated with a surface aerator type BSK-Gigant. Calculations were made for 10 measurements series, at the assumption of the following values of the model's parameters:

$h = 1.0 \text{ m}$, $P = 320 \text{ sq. m}$, $z = 1.5 \text{ m}$, $z_0 = 0.04$ - meadows, $u_p = 0.003 \text{ m ms}^{-1}$, $\lambda = 0$. - for TVP. Value of the TVP emission indicator is $29890 \text{ microorganisms m}^{-2}\text{s}^{-1}$.

Object D

Sewage aerating chambers are the chief source of the emission of microbes. In the course of the research five chambers were used, in which there were installed three surface aerators in each. The calculations were made for the following parameter values:

$h = 1.8 \text{ m}$, $P = 4680 \text{ sq. m}$, $z = 0.8 \text{ m}$, $z_0 = 0.06 \text{ m}$, $u_p = 0.003 \text{ m s}^{-1}$, $\lambda = 0.5^{-1}$ - for TVP.

Average value of the emission indicator is $11740 \text{ microorganisms m}^{-2}\text{s}^{-1}$. The value is more than two times lower, than the intensity of small objects emission. The above proves the fact, that the emission of microbiological aerosol from large areas is much limited by the particles re-fall down on the liquid surface.

Object E

The investigations were conducted around sedimentation lagoons of the area of $50\,000 \text{ sq. m}$. The lagoons serve dewatering of sludge in municipal sewage treatment plants, to which there had been raw sewage ducted from the fibreboard production factory. In the result of microbiological examination it was found, that concentrations on the leeward side of the lagoons oscillate around concentration values on the windward side. It means, that average values of TVP concentration increase are close to zero, and that the object is no significant source of microbiological emission. At the same time, chemical analysis showed occurrence of significant emission of ammonia ($0.30 \text{ mg m}^{-2}\text{s}^{-1}$), and hydrogen sulfide (about $0.01 \text{ mg m}^{-2}\text{s}^{-1}$).

C O N C L U S I O N S

On the ground of the measuring-calculating investigations and analysis of the obtained results the following conclusions were drawn:

1. The suggested method of determining values of microbiological pollutants emission is relatively simple, and at the same time it takes into account all the substantial factors effecting range of emission, and the condition of the atmosphere pollution.
2. Value of emission obtained through the application of the presented method is calculated by the quantity of microbes carried to the atmosphere in a time unit, and the quantity contributes to the degree of the atmosphere's pollution, the above being determined by measurements.
3. In case there occur various emission sources in the object, one should drive at the isolation of emitters of uniform emission distribution. Such a procedure produces better results, than the use of one substitute source of emission for the whole object (e.g. object B).

4. Aeration chambers are the main sources of the emission of microbiological pollutants in sewage treatment plants. The emission of microorganisms depends directly on their concentration in the liquid being aerated as well as on aerator working parameters, area of emission and meteorological conditions. In majority of cases the emission values grow up when the unstability of the atmosphere increases.

5. The above presented attempt to determine factors influencing the value of emission of microbiological pollutants from the sewage treatment plant does not exhaust the problem. It requires further detailed research on all the conditions of atmospheric stability, and a wider range of wind velocity, and simultaneous monitoring of process parameters of the investigated object.

A P P E N D I X

The following formula has been applied to determine the value of microbiological pollutants emission:

$$S(x, y, z) = \frac{E}{2\pi \bar{u} \sigma_y \sigma_z} \exp \left[-\left(\frac{y^2}{2 \cdot \sigma_y^2} \right) \right] \cdot \left\{ \exp \left[-\frac{(z + u_p \cdot t - H)^2}{2 \cdot \sigma_z^2} \right] + f(u) \exp \left[-\frac{(z + u_p \cdot t + H)^2}{2 \cdot \sigma_z^2} \right] \right\} \exp(-\lambda \cdot t) \quad (1)$$

where: $S(x, y, z)$ - microorganisms concentration (in numbers) at the measurement points of rectangular coordinates x, y, z , direction of axis x coincides with the wind direction (quantity/ m^3),

E - emission intensity in numbers (quantity/s),

$\bar{u}$ - average wind velocity in the air layer from $z = 0$ to $z = H$ (m/s),

H - effective height of emitter (m),

σ_y, σ_z - coefficient of horizontal and vertical atmospheric diffusion of Pasquill type (m),

u_p - deposition velocity of bioaerosol (m/s),

λ - death rate of microorganisms (1/s),

t - biological aerosol age (s),

$f(u)$ - function of pollutants reflection from the ground surface (-).

Assuming exponential profile of wind velocity, average wind velocity in the layer is expressed by the formula:

$$\bar{u} = \frac{u_a}{m+1} \cdot \left(\frac{H}{h_a} \right)^m \quad (2)$$

where: u_a - wind velocity at the height of anemometer h_a (m/s),

m - meteorological exponent dependent upon condition of thermodynamic stability of atmosphere (-).

Considering the fact, that microbial pollutants emission does not take place from a point emitter, but from the surface, an apparent distance of virtual emitter x_y has been introduced to the formula of the coefficient of horizontal atmospheric diffusion:

$$\sigma_y = A \cdot (x + x_y)^a \quad (3)$$

Coefficient of the vertical atmospheric diffusion is expressed by the formula:

$$\sigma_z = B \cdot x^b \quad (4)$$

Constants in formulas 3 and 4 distance x_y have been calculated as follows:

$$A = 0.08 \cdot (6 \cdot m^{-0.3} + 1 - \ln \frac{H}{z_0}) \quad (5)$$

$$B = 0.38 \cdot m^{1.3} (8.7 - \ln \frac{H}{z_0}) \quad (6)$$

$$a = 0.367 \cdot (2.5 - m) \quad (7)$$

$$b = 1.55 \cdot \exp(-2.35 \cdot m) \quad (8)$$

$$x_y = (\sqrt{P/\pi} / A)^{(1/a)} \quad (9)$$

where: z_0 - aerodynamic surface roughness length (m),

P - area of emitter (sq. m).

The reflection function has been made dependent on wind velocity, assuming

$$f(u) = \frac{u_a}{7} \quad \text{for } u_a \leq 7 \text{ m/s} \quad (10)$$

$$f(u) = 1 \quad \text{for } u_a > 7 \text{ m/s} \quad (11)$$

Age of biological aerosol in the measurement point is dependent on the average wind velocity and is expressed:

$$t = \frac{x}{u} \quad (12)$$

Upon substitution of dependence 2-12 in the formula 1 and upon its transformation in relation to value E there has been obtained a formula for average emission equivalent to one series of sampling.

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Use of moss bags to monitor air pollution in Vantaa, southern Finland

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Moss (*Sphagnum*) bags have been used successfully in Finland to study the distribution and deposition of heavy metals around point sources of pollution. This paper presents results concerning their use to study the deposition of lead around a lead smelter in Vantaa, southern Finland. Contents of lead collected by moss bags have been analyzed and the results calculated as a function of time and the results are also compared to those using other methods.

Using the moss bag method a good view was obtained about the distribution of lead in the environment, the change in the smelting process could be clearly seen in the accumulation of lead into the moss bag, and correlations between the metal contents in moss bags and the deposition values given by other methods were mostly high.

It was found that the moss bag method is rapid, inexpensive and easy to use in monitoring the emissions from point sources of pollution. It enables a dense monitoring net compared to the standard collectors. The method is not confined to the existence of mosses growing in situ. However, moss bags must be properly standardised before use.

INTRODUCTION

Mosses growing in situ have long been used in the monitoring of air quality (e. g. LOTCHERT et al., 1975; MÄKINEN, PAKARINEN, 1977; ANDERSEN et al., 1978). However, very often it is not possible to find suitable moss stands around point sources of pollution as they are situated on industrial areas. In such cases transplant methods may be used, of which the moss bag method is one. The method was originally developed in Great Britain (GOODMAN et al., 1974; LITTLE, MARTIN, 1974; TEMPLE et al., 1981) where it has recently been intensively used again (GAILEY, LLOYD, 1986a, b, c, d). In Finland, the method has been used since 1976 for air pollution monitoring in cities and near industrial and traffic emission sources (MÄKINEN, 1977, 1987; KINNUNEN et al., 1985; HYNINEN, 1986). The method has also been used to collect background air pollution data in relation to the distribution of forest insect pests around point sources of pollution (e. g. HELIOVAARA, 1986; HELIOVAARA, VÄISÄNEN, 1986). The first studies about lead pollution in the city of Vantaa in south Finland were made in 1968 - 1971 by the Agricultural Research Centre when the effects of a lead smelter on lead deposition with snow, on contents in field and forest soils, and crops were studied (LAKANEN, ERVIO; LAKANEN, 1973). Lead emissions from traffic were found to have no significance compared to that from the smelter.

In the beginning of the 1980s, lead contents in crops and vegetables grown near two lead smelters within the city limits exceeded the limits set for food, and the health care authority gave out cultivation restrictions near the smelters. Studies on lead deposition have continued using food plants, snow, mechanical collectors and moss bags (LEINONEN, MIKKOLA, 1985; VASANDER, MIKKOLA, 1986; MIKKOLA, 1987).

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STUDY AREA

Vantaa is a rapidly growing city with 150 000 inhabitants neighbouring Helsinki to the south. The city consists of several centres of population among an essentially rural landscape. Main roads from Helsinki to other parts of Finland pass through the city, and the main airport of Finland is located in Vantaa. A lead industry has been established in the area before the city began to rapidly grow in 1960s and 1970s. In the beginning of 1980s there were one factory producing car batteries and two lead smelters at Vantaa.

We monitored accumulation of lead in the vicinity of the Bera lead smelter situated near the airport and beside two busy main roads. The average daily traffic load of these two roads was 20 000 vehicles in the beginning of 1980s. The amount of traffic on an area of 8 km^{-2} around the smelter is approximately 120 000 km/24 h (VANTAAN KAUPUNKI, 1983).

The smelter mainly used disused car batteries and other leadcontaining waste. The lead waste was first crushed and smelted in a pit-furnace before being refined. The annual amount of lead waste smelted in the beginning of 1980s was approximately 3 600 tons, of which 3 000 tons came from disused car batteries, 400 tons from metal lead waste, and 100 - 200 tons from leadcontaining clinker and ash (PESONEN, 1983).

Lead emissions from the pit-furnace smelting process have been found to be several times higher than from the refining process. Lead emissions were $30 - 170 \text{ kg Pb mo.}^{-1}$ when the pit-furnace smelting was on after yearly maintenance of filters. These are minimum values as emissions could not be measured from all possible sites (PESONEN, 1983). Pit furnace smelting ceased in March 1984 and refining fell by 70 %.

Deposition in the surroundings of the smelter had been investigated since the 1970s using deposition collectors. In 1973 - 1974 the mean deposition of lead 100 m from the smelter was $515 \text{ mg Pb m}^{-2} \text{ mo.}^{-1}$ ($n = 11$), and 300 m from the smelter $35 \text{ mg Pb m}^{-2} \text{ mo.}^{-1}$ ($n = 10$). In 1976 - 1977, the values were 52 ($n = 4$) and 15 ($n = 8$) $\text{mg Pb m}^{-2} \text{ mo.}^{-1}$ at 100 and 400 m, respectively. At other sites in the city, values ranged between 1 and 3 $\text{mg Pb m}^{-2} \text{ mo.}^{-1}$ (MIKKOLA, 1987).

Positive changes in the lead pollution of Vantaa have occurred since the beginning of 1980s. The other lead smelter ceased functioning in 1984. The lead content of gasoline was lowered from 0.4 to 0.15 g Pb/l in 1984 for low octane and in 1985 for high octane gasoline in the whole country. These three events (cease of one smelter, cease of pit-furnace smelting in the other and lower lead content in gasoline) have lowered the lead deposition in Vantaa to approximately half of the corresponding annual mean value in the beginning of 1980s being now $0.7 - 0.9 \text{ mg Pb m}^{-2} \text{ mo.}^{-1}$ (MIKKOLA, 1987).

MATERIAL AND METHODS

Moss bag technique

Sphagnum girgensohnii Russ. was gathered from a remote area in middle Finland. The species belongs to the Acutifolia section (cf. ISOVIITA, 1966) which has a higher cation exchange capacity than Cuspidata and Palustria sections (e. g. PUUSTI-JÄRVI, 1956). The species also forms large pure stands in paludified forests and spruce mires enabling easy collection.

In the laboratory, the moss was rigorously washed to remove extraneous material. Only green shoots were selected, thereby excluding the decaying shoots in which cation exchange sites had been at least partly destroyed. The moss was then acid washed with 0.5 M HCL after which it was washed several times with deionized water. Rigorous cleaning and washing of the material is necessary for good functioning of the method. An amount of the moss, about 1 g d.w., was put into a nylon chignon net in the form of sphere. This amount of moss has been usually used in Finnish studies (HYNNINEN, 1986; MÄKINEN, 1987) and elsewhere (e. g. CAMERON, NICKLESS, 1977), although GAILEY et LLOYD (1986a) have recently recommended a smaller size (0.1 - 0.2 g d. w.).

The moss bags were suspended at about 2 m height at 33 locations along five study transects and in a background area situated 12 km north-west from the smelter. There were 3 replicates at each location. The balls were suspended from birch branches (leafless period) or wooden poles on field areas. The hanging time was two months from October-November to December-January in 1983 - 1986.

After exposure, the moss bags were transported to the laboratory in plastic bags. The moss was removed from the nylon net containers and oven-dried at $+65^{\circ}\text{C}$ to constant weight. The mosses were wet-digested with HNO_3 and lead contents were analyzed by using AAS, and graphite oven in the case of small contents. Lead contents were calculated as ppm Pb per month. Lead content of non-exposed bags was reduced from the values of exposed bags. Weather data are from Helsinki-Vantaa airport, 2 km north from the study area.

Comparison to other methods

Some moss bags were also suspended beside standard deposition collectors (SFS 3865) at five sites situated 1.5 - 9 km from the smelter. Deposition ($\text{mg Pb m}^{-2} \text{ mo.}^{-1}$) was measured monthly.

Lead deposition was also monitored using vertical snow sampling (e. g. JERNELÖV, WALLIN, 1973; SOVERI, 1985; ETTALA et al., 1986) in March 1984 - 1986 before the snow started to melt. Samples were taken with a 1 m long transparent PVC-tube (8 cm in diameter) from the top of the snow cover to the soil surface and the values calculated as $\text{mg Pb m}^{-2} \text{ mo.}^{-1}$. Although snow samples were taken near moss bag locations the sampling periods were not the same.

Lettuce leaves collected from 150 - 1 300 m from the smelter in different directions since 1981 have also been analyzed. Naturally contents represent accumulation during the summer.

R E S U L T S

Lead accumulation clearly decreased with increased distance from the smelter (Fig. 1). In 1983 the accumulation values were clearly higher than in 1984 when pit-furnace smelting was discontinued. In 1983 several accumulation values over $1\,000 \text{ ppm mo.}^{-1}$ were recorded near the smelter, the maximum being $2\,400 \text{ ppm mo.}^{-1}$. In 1984 the maximum value near the smelter was 390 ppm mo.^{-1} (Fig. 1). In 1985 and 1986, the maximum accumulation values were approximately 200 ppm mo.^{-1} (App. 1). Changes in the directions of lead distribution between years may, however, be due to the differences in wind directions. Background values measured in northern Vantaa were 4 ppm mo.^{-1} . Variation between replicates was generally small, the median coefficient of variation being 15 %

There was a high correlation between the lead accumulation as measured by the moss bags and the standard deposition collectors (Fig. 2) and snow sampling (Fig. 3). The form of the equation and the correlation coefficient were different when the pit-furnace was in use. Accumulation values were clearly smaller and the value of the correlation coefficient higher after pit-furnace smelting ceased.

The cessation of pit-furnace smelting was also well indicated by the snow samples. During the 1983 - 1984 winter the maximum value was $90 \text{ mg Pb m}^{-2} \text{ mo.}^{-1}$ in 1984 - 1985 $38 \text{ mg Pb m}^{-2} \text{ mo.}^{-1}$ and in 1985 - 1986 $21 \text{ mg Pb m}^{-2} \text{ mo.}^{-1}$. The correlation between snow deposition and the accumulation of lead into the moss bag was good for each studied transect (Fig. 3). If the snow deposition values during pit-furnace smelting were included the variation had become greater and the correlation less tight. During uneven emissions (pit-furnace smelting) winds have greater effect on deposition than during more even emissions.

The relationship between lead deposition measured by standard collectors and vertical snow sampling was good:

$$y = -0.01 + 0.61x, n = 6, r = 0.98.$$

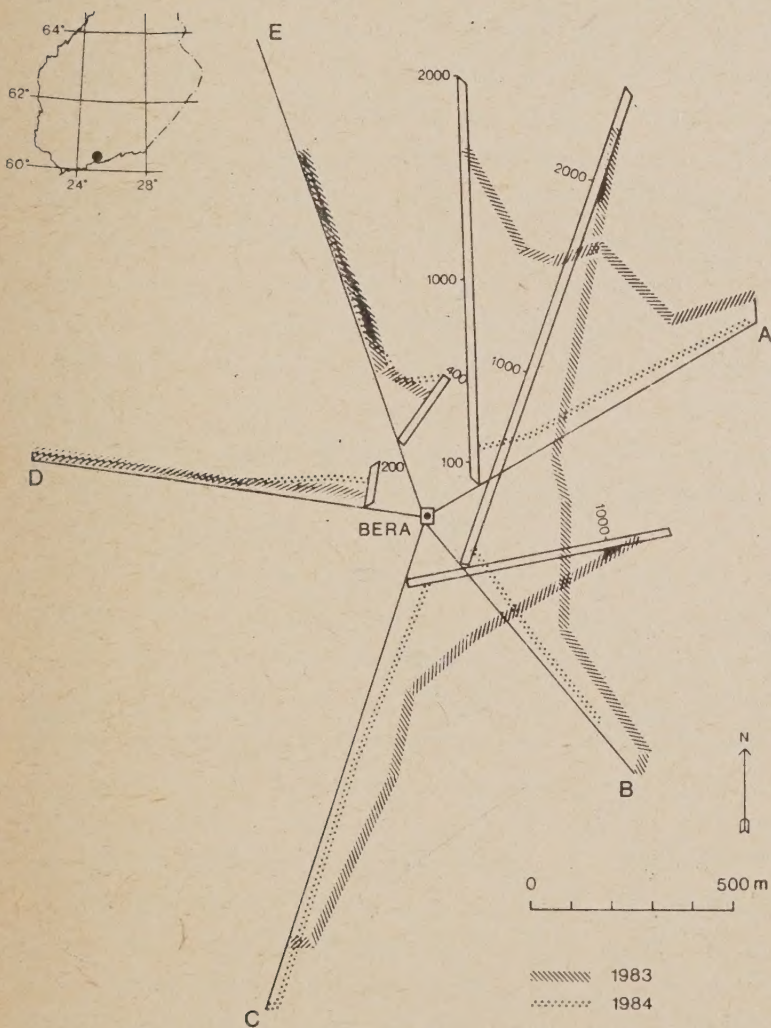


Fig. 1. The rates of lead accumulation (ppm Pb per month, z-axis) for each study transect in 1983 (during pit-furnace smelting) and in 1984 (after cessation of pit-furnace smelting).

The lead content in lettuce leaves near the smelter clearly decreased after the cessation of pit-furnace smelting. But even afterwards, values exceeded the limits set by the Ministry of Trade and Industry in 1985 (0.5 ppm for leaf vegetables and 0.2 ppm for other vegetables). Lettuce leaf lead concentrations varied rather much between different years (Tab. 1).

DISCUSSION

The moss bag method allows one to calculate the accumulation time more exactly the when using indigenous moss samplers. The shape of the spherical moss bags allows them to collect metal particles relatively equally from all directions. Thus metal concentrations in them are higher and more consistent than those in flat moss bags (GAILEY, LLOYD, 1986c). *Sphagnum* moss bags have been successfully used because of several advantages (GAILEY, LLOYK, 1986d). These include:

- a) higher proportion of cation exchange sites compared to other plant species (CLYMO, 1963),
- b) the large amount of leaves are arranged spirally around the stem enhancing the capture of particles from the ambient atmosphere,
- c) due to the large number of dead hyaline cells the moss can hold a water volume which exceeds its structural weight by more than 20-fold,
- d) it can absorb cations from very dilute solutions such as rainwater.

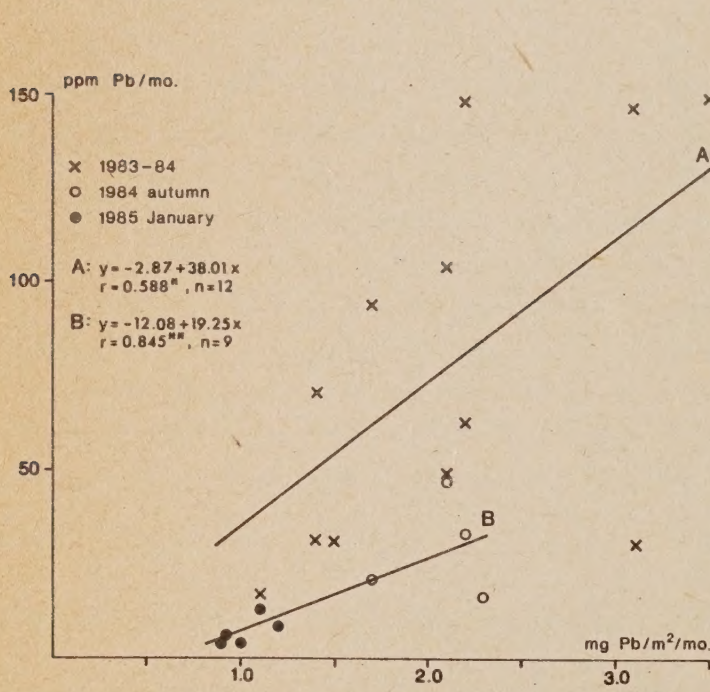


Fig. 2. The relationship between the rate of lead deposition measured by the moss bag method and the standard collectors. The relationship has been determined separately during the time of pit-furnance smelting (A, crosses) and after it ceased (B, points).

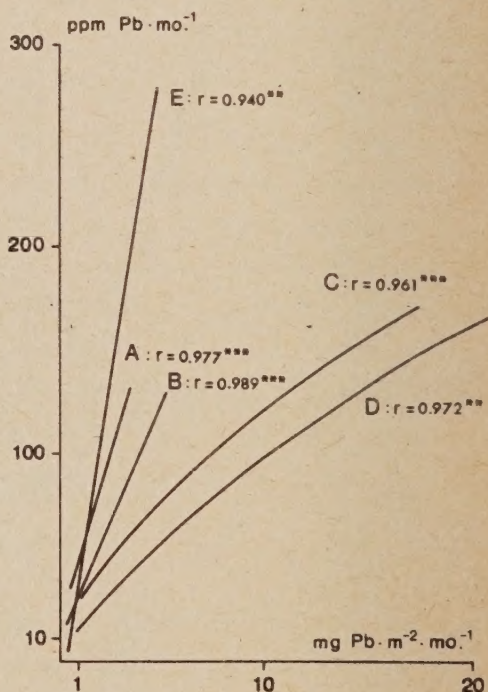


Fig. 3. The relationship between the rate of lead deposition measured by the moss bag method and the vertical snow sampling for each study transect (cf. Fig. 1).

Tab. 1. Lead concentrations (ppm Pb) in lettuce leaves from different sites around the smelter in 1981 - 1986. - no data.

Distance and direction	1981	1982	1983	1984	1985	1986
150 m N	5.7	1.4	4.1	1.2	1.3	1.3
350 m SW	-	-	0.36	-	0.36	0.32
650 m E-NE	0.18	0.04	0.20	0.18	0.24	0.18
700 m SW	0.36	0.04	0.24	0.13	0.74	0.42
1080 m NE	0.26	0.06	0.37	0.18	0.17	0.48
1300 m E-NE	0.22	0.17	0.20	0.69	0.41	0.34

In calm and weakly windy weather, the moss bags absorb mainly dry deposition, e. g. dust. As the humidity increases, the moss bags can absorb a great quantity of water and particles are adsorbed by the hyaline cells, as in living mosses. When the weather again dries, water is evaporated leaving the particles absorbed in the moss (MÄKINEN, 1984).

The accumulation values now obtained may be compared to those caused by traffic along main roads. The highest accumulation value recorded a few meters from the edge of one of the roads was 310 ppm Pb mo.^{-1} . At 100 m distance, the highest accumulation value was 30 ppm Pb mo.^{-1} . Thus, the present lead emissions from the smelter now result in accumulation similar to that near main roads and that of car battery production. Lead deposition near a car battery factory in the nearby city of Espoo was 340 ppm Pb mo.^{-1} (KINNUNEN et al., 1985). Emissions from pit-furnace smelting, however, clearly exceeded that of traffic.

Background lead deposition values in northern Vantaa (4 ppm mo.^{-1}) are similar to those recorded in the neighbouring city of Espoo and in more remote areas (KINNUNEN et al., 1985; MÄKINEN, 1987). Also noteworthy is that the method worked well during cold winter time (Fig. 2, also HYNINEN, 1986). The moss bags were not saturated with lead (LEINONEN, MIKKOLA 1985).

The moss bag method is very suitable to study the distribution of emissions from point sources of pollution. However, the moss bags should be calibrated if absolute deposition data are required. The correlation between deposition collector values and moss bag values was better after pit-furnace smelting had ceased. Probably the heaviest lead particles are not accumulated well by the moss.

Although the monitoring periods used for snow deposition collection and moss bag accumulation only partly overlapped, the correlation between the methods was good. The curvilinearity of the relationship with higher values was probably due to the inability of moss bags to accumulate large lead particles. ETTALA et al. (1986) reported similar high correlations when they compared the snow deposition of chrome and the concentration of chrome in mosses (*Hylocomium splendens* (Hedw. B.S.G.) around a steel factory in northern Finland. Also in their study the sampling periods were only partly overlapping. The correlation between the standard collectors and snow core samples was tight.

No correlation was calculated between lead accumulation measured by the moss bags and lead contents in lettuce leaves; there were too few data for this. There were, however, some samples exceeding the limits set for food-plants even after the cessation of pit-furnace smelting. Probably lead accumulated in the soil, rain showers and winds (lead dust) could explain this

variation. Although lettuce leaves are not suitable to study the deposition of lead, the amount of lead people consume in food is extremely important because of the health hazards.

CONCLUSIONS

The moss bag method is an easy, rapid and cheap technique for monitoring the emissions from point sources of pollution, enabling a dense monitoring network. The method is not confined to the existence of mosses growing in situ. However, moss bag method must be properly standardised before use (single moss species, rigorous cleaning and washing, size of the bag, exposure time).

Appendix 1. The rates of lead accumulation (ppm Pb mo.^{-1}) measured by the moss bags for each study transect in 1983 - 1986. Dist. = distance from the smelter (m), - = no data.

Transect A (62°)					Transect B (133°)				
Dist.	1983	1984	1985	1986	Dist	1983	1984	1985	1986
150	1600	160	100	190	150	2400	110	160	130
300	970	88	71	64	280	790	53	59	61
360	880	66	35	74	350	670	36	23	21
500	800	61	31	-	480	180	20	23	33
700	220	29	17	17	680	150	15	15	30
940	93	29	16	16	820	100	16	10	22
1160	-	28	-	-					
Transect C (207°)					Transect D (274°)				
170	1200	120	200	76	160	84	119	170	31
270	-	-	110	66	260	78	118	110	14
350	440	110	80	26	360	40	-	63	-
470	200	74	43	20	490	42	27	17	-
700	240	35	49	13	660	61	29	14	13
1010	110	39	29	19	1000	16	13	13	8
1280	-	61	26	51	1280	-	14	-	-
Transect E (336°)									
190	270	390	150	230					
280	160	130	120	61					
360	85	120	59	37					
490	80	28	26	15					
640	31	18	22	-					
950	25	8	-	-					
1260	-	8	3	5					

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Effect of local air pollution on element concentration in soil and sessile oak leaves in the vicinity of industrial districts

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INTRODUCTION

The effect of the wet or dry deposition of anthropogenic air pollutants on the vegetation and the soil has been intensively studied all over the world in recent times (SMITH, 1981; ULRICH, PANKRATH, 1983; HUTCHINSON, HAVEAS, 1980).

In searching for the causes of new types of forest diseases and tree decay the European and North American specialist literature gives prominence to the approach which focuses attention to the changed element concentration in the leaves of the diseased trees and in the soil under the trees. The majority of the studies are primarily concerned with the trees of coniferous forests (HUTTLE, ZÜTTLE, 1985), since impairment of such forests is of higher economic significance in Europe and America, and further, these are the predominant trees in the forests of these territories (ZÜTTLE, MIES, 1983). Investigations on the element concentration of deciduous forests are reported on by BREDOV et al. (1983) and GLAVEC (1987).

The new types of fast-action forest diseases and tree decay are signalled by changes in element concentration in the soil and the assimilative organs of the trees.

In Hungary during the past ten years the heaviest destruction afflicted the individual specimens and forests of sessile oak (Quercus petraea). Previous investigations (JAKUCS et al., 1986) suggest that the primary impairing factor for deciduous trees is the effect of air pollution inducing acidification of the soil, and, through this effect, resulting in changes in the element concentration of the soil. The process of increasing changes in the soil is manifested in the ion uptake of the trees and, thus, in the changes in element concentration in the leaves of the foliage.

The impact of the air pollutants capable of covering longer distances (SO_2 , NO_x) can be greatly enhanced by the excess emission appearing as addition to the normal value in the immediate vicinity of industrial areas.

In 1987 our Institute performed complex investigations in a large industrial district in Northern Hungary, the Sajó-valley, to reveal the local effect of the emitted air pollutants. In this study several abiotic and biotic parameters were studied jointly and concomitantly, continuously moving farther away from the points of emission (JAKUCS et al., 1988; HOLES, BERKI, 1986; JAKUCS, BABOS, 1988).

The element content examination of the tree leaves and the soil was considered important since, in the opinion of FIEDLER et al. (1973), sessile oak is the species of highest nutrient demand among the oak species, and it was assumed that it reacts sensitively to changes in nutrient element content due to air pollution.

The objective of the present study is to summarize the results of the chemical analyses of the soil and the leaf.

METHOD AND MATERIAL

The Sajó-valley industrial district is situated in the North-eastern part of Hungary (Fig. 1). To detect the effect of local air pollutants originating in the industrial district, on the soil and the plant, soil and leaf samples were taken from 8 forest stands growing on the hills Southwest of the Sajó-valley, in the period between July 13th-17th, 1987. The stands were located in a circle of 0.5 - 12 km from the industrial emission sources and tree decay was much greater here than the nationwide average.

In the same period samples were taken from 3 stands at a distance of 15- 25 km from the industrial district. These samples were used as controls, however, in the evaluation we made use, on several occasions, of results from 5 control forest stands from a study performed between July 20th-24th, 1987, in the Western part of Hungary, which are situated far from any kind of greater industrial emission (Fig. 1). The control stands were healthy and hardly and diseased trees were found in them.

Soil samples were taken in the stands under study from three genetic soil horizons ($A_1 = 0 - 10$ cm; $AB = 10 - 30$ cm; $B_1 = 30 - 50$ cm). Then after felling the trees above the soil-sampling places, mixed leaf samples (upper-medium-lower levels) were taken from their foliage. At the places near the industrial district, where there were healthy and diseased trees standing close to one another, the samples were taken from pairs of trees, however, this study gives data primarily on the healthy trees. This procedure is justified by the fact that in the control forests there were hardly and diseased trees.

The examinations were concerned with the parameters and elements included in Tab. 1 and 2.

The symbol y_1 (hydrolytic acidity) denotes $0.5 \text{ mol/L Ca (CH}_3\text{COO)}_2$ adjusted to pH 8.0, Y_2 (exchangeable hydrogen) the titratable potential acidity released through separation by shaking out with 1 mol/L KCl solution.

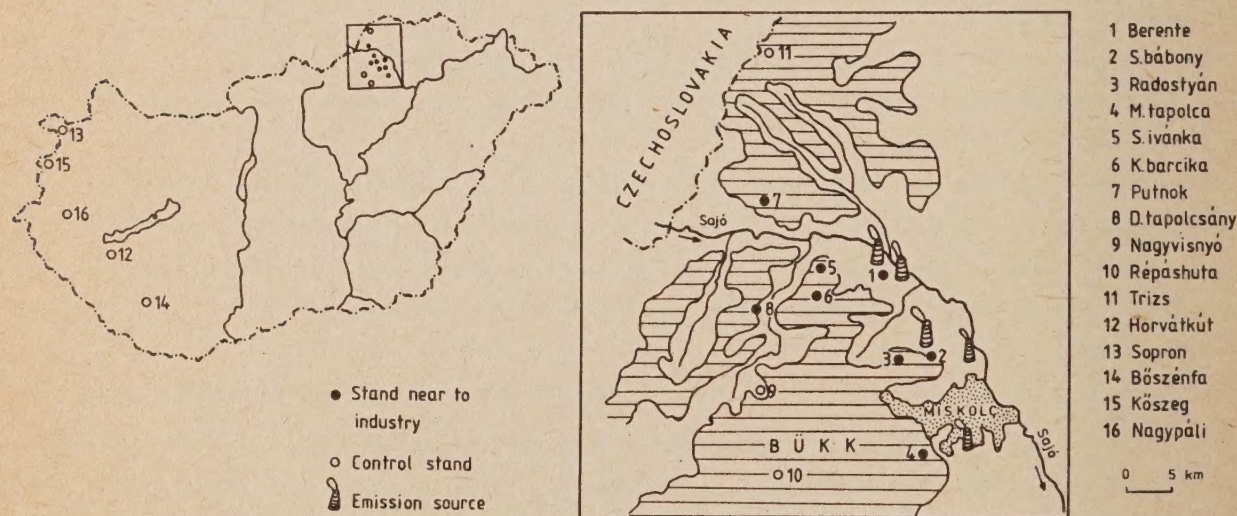


Fig. 1. The location of the examined sessile oak stands in the country and in the vicinity of the Sajó-valley industrial district.

Tab. 1. Mean values of the soil analyses of sessile oak stands under the healthy trees (in the 10 - 30 cm soil horizon)

	Stands near to industry (n = 7)		Control stands (n = 7)	
	$\bar{x}$	s	$\bar{x}$	s
pH (H ₂ O)	3.91	+0.28	4.47	+0.50
pH (KCl)	3.24	+0.17	3.89	+0.67
Y ₁	42.16	+14.14	24.39	+9.60
Y ₂	28.84	+15.74	17.30	+4.30
Humus %	1.67	+0.32	1.92	+1.08
NO ₃ ⁻ -N mg/kg	7.76	+7.28	0.81	+0.45
NH ₄ ⁺ -N mg/kg	4.09	+1.56	12.92	+8.48
P ₂ O ₅ mg/kg	42.14	+64.96	17.57	+22.66
K ₂ O mg/kg	143.3	+56.96	116.1	+33.09
SO ₄ ²⁻ -S mg/kg	22.54	+13.61	17.38	+10.16
Ca mg/kg	254.9	+213.9	425.6	+500.5
Mg mg/kg	68.43	+49.98	123.1	+89.28
Mn mg/kg	343	+203.3	492.8	+370.4
Na mg/kg	26.57	+11.16	29.70	+15.32
Fe mg/kg	481.1	+204.6	444	+137.1
Al mg/kg	843	+359.1	494.7	+246.6
Zn mg/kg	2.20	+0.98	2.29	+1.19
Cu mg/kg	2.06	+1.17	1.85	+0.70
B mg/kg	0.806	+0.25	0.734	+0.45
Mo mg/kg	0.130	+0.09	0.039	+0.03
Ni mg/kg	1.68	+1.40	1.7	+0.88
Li mg/kg	0.089	+0.06	0.058	+0.06
Co mg/kg	5.77	+2.44	5.68	+2.59
Cr mg/kg	0.130	+0.08	0.186	+0.10
Pb mg/kg	10.97	+1.96	11.02	+2.86
Cd mg/kg	0.056	+0.02	0.056	+0.03
Ca/Al	0.29	+0.26	0.86	+0.59

Dissolution of NO₃⁻, NH₄⁺, SO₄²⁻ and Mg from the soil was performed with 1 mol/l KCl separating agent, the extraction of P, K, and Na with ammonium-lactate (AL) solution. The detection of the rest of the elements included in Tab. 1 took place by EDTA extraction, with an EDTA concentration of 0.05 mol/l and 0.01 mol/l KCl.

Exposure for the determination of the N, P, K and P content of the leaves was performed with concentrated H₂SO₄ + H₂O₂, that of the other elements with HNO₃ + H₂O₂. The measurements for humus, NO₃⁻, NH₄⁺, SO₄²⁻ and P were carried out with a Type OL 603 photometer, for K and Na with an OM. Sz8v. Type OE 85 flame photometer, for all the other elements with a UV-25 Lab-test ICP spectrometer.

RESULTS OF THE SOIL ANALYSES

Out of the 16 stands of sessile oak the soil of 12 was loamy lessivated brown forest soil evolved on Tertiary sediment, that of 4 acidic, non-podzolised brown forest soil evolved on slate. The most important of the 3 soil horizons studied, as to the nutrient uptake of the trees, was the AB horizon at 10 - 30 cm, since the majority of the thin root system of sessile

oak can be found here. In this way, Tab. 1 and Figs. 1 - 5 present the mean values of the element content of this horizon.

In the following those soil parameters are evaluated that may play a role, as shown by the results obtained, in the evolution of oak-tree decay accelerated by local industrial air pollution.

In Table 1 one can see that the soil of the stands under examination is strongly acidic. At the same time, one can also observe that the two acidity values (y_1 and x_2) and the two different pH values (measured for H_2O and KCl) unequivocally show acidification of soils near the industrial districts with respect to those of the control stands. The mean pH (KCl) value in the AB horizon in the diseased oak stands near the industrial zone is only 3.24!

The stronger acidity of the soils in the near-industry stands is evidence for soil acidification due to industrial air pollution, since out of the 8 control locations the soil of 4 is acidic, non-podzolised, brown forest soil, originally highly acidic owing to its genetics. On the other hand, the near-industry stands lie, in every case, on lessivated brown forest soils, which cannot have been originally as acidic as the acidic, non-podzolised, brown forest soils.

In correlation with the soil acidity conditions one can assess the readily soluble Ca and Al content of the soil. The absolute amounts of these two elements, especially their quantitative proportions (Ca/Al ratio), indicate the pH conditions of the soil as well. In the whole region, corresponding to the low pH, the soil of the control areas contains, on the average, 425.6 mg/kg Ca, whereas this value for the near-industry stands is 254.9 mg/kg Ca in the same soil horizon. In the soil of the near-industry stands, corresponding to the lower pH as compared to the controls, the soluble Al content is nearly 50 % higher (843 mg/kg) than in the soil of the control areas (495 mg/kg). Consequently, the Ca/Al ratio in the soil of the near-industry stands is on the average, only 32 % (0.29) with respect to the soils of the control stands (0.86).

In the near-industry stands the NH_4^+ content of the soils was only one third of the value found in the control soils, whereas the NO_3^- value was nearly ten times as high near the industrial zone as in the non-industry areas. This excess of NO_3^- may in part be due to the nitrous gases emitted by the nearby factories.

RESULTS OF LEAF ANALYSES

The mean P content in the leaves of sessile oaks near the industrial areas was 0.146 %, the mean of the controls 0.204 % (Tab. 2, Fig. 2). The mean concentration of P is low not only with respect to the controls but in absolute value too. Experiments have proved (NEWMAN, CARLISLE, 1969) that most of the production of sessile oak is attributed to the 0.22 % P content of its leaves.

In the vicinity of industrial areas the amount of K is also decreased in the leaves (Tab. 2, Fig. 3). In the repeated acerous-leaf analysis of pinewoods in Southern Germany in the course of 20 years it was established that during these two decades the K content of pine trees has greatly decreased. The authors consider deficiency in K as one of the eliciting factors of damage symptoms (ZÖTTL, HUTTL, 1985).

Tab. 2. Mean values of the leaf analyses of sessile oak stands in the case of healthy trees
(referred to dry material content)

	Stands near to industry (n = 8)		Control stand (n = 8)	
	$\bar{x}$	s	$\bar{x}$	s
N %	2.105	+0.22	1.89	+0.23
P %	0.146	+0.02	0.204	+0.02
K %	0.768	+0.11	1.09	+0.11
S mg/kg	1710.0	+234.5	1310.0	+208.7
Ca mg/kg	5520.0	+1304.3	6966.0	+1008.7
Mg mg/kg	1312.0	+346.3	1685.0	+326.7
Mn mg/kg	1834.0	+434.7	1399.0	+701.3
Na mg/kg	312.0	+39.72	262.0	+67.46
Fe mg/kg	375.4	+156.2	155.2	+22.52
Al mg/kg	140.0	+28.85	85.4	+5.69
Zn mg/kg	18.98	+1.78	16.85	+3.21
Cu mg/kg	8.21	+1.02	7.55	+1.27
B mg/kg	29.66	+5.42	19.93	+4.60
Mo mg/kg	0.25	+0.51	0.118	+0.08
Ni mg/kg	7.56	+9.35	4.58	+1.79
Li mg/kg	0.31	+0.08	0.064	+0.06
Co mg/kg	0.433	+0.37	0.171	+0.24
Cr mg/kg	10.08	+22.24	2.69	+1.64
Hg mg/kg	7.71	+2.37	5.59	+2.58
Pb mg/kg	2.09	+0.58	0.643	+0.20
Cd mg/kg	0.162	+0.08	0.138	+0.06
N/P	15.43	+4.35	9.38	+1.49
N/K	2.81	+0.63	1.63	+0.45
Ca/Al	41.11	+13.12	81.57	+10.6

The lower P and K content of the leaves in the near-industry stands cannot be accounted for by the lack of these elements from the soil, since these elements are present in the soils of these stands, too, in at least identical amounts as in the control stands (Tab. 1, Figs. 2 and 3).

The studies concerned with the nutrient uptake of the trees mention that the symbiont mycorrhizal fungi promote the phosphorus uptake of the roots (MEYER, 1962; AGERER et al., 1968). In the sessile oak stands near the Sajó-valley industrial district, the mycorrhizal associations on the roottips show degradation with respect to the control stands (HOLES, BERKI, 1988). On this basis one can assume that the lower P content of the leaves in this stand is in connection with the near-industry damage of the root-mycorrhiza association, too.

There is also less Ca and Mg in the leaves in the stands near the Sajó-valley than in the control areas (Tab. 2).

In contrast to the elements mentioned so far, in the leaves of the near-industry stands the S, Al, Mn and Fe content is higher than in the control ones (Tab. 2, Figs. 4 and 5).

As to the excess of Al, Mn and Fe in the leaves, it is only the excess of Al that can be accounted for by the higher Al content of the soil in the area near the industrial district, since the Mn and Fe content of the soil is not higher in this region than in the control stands.

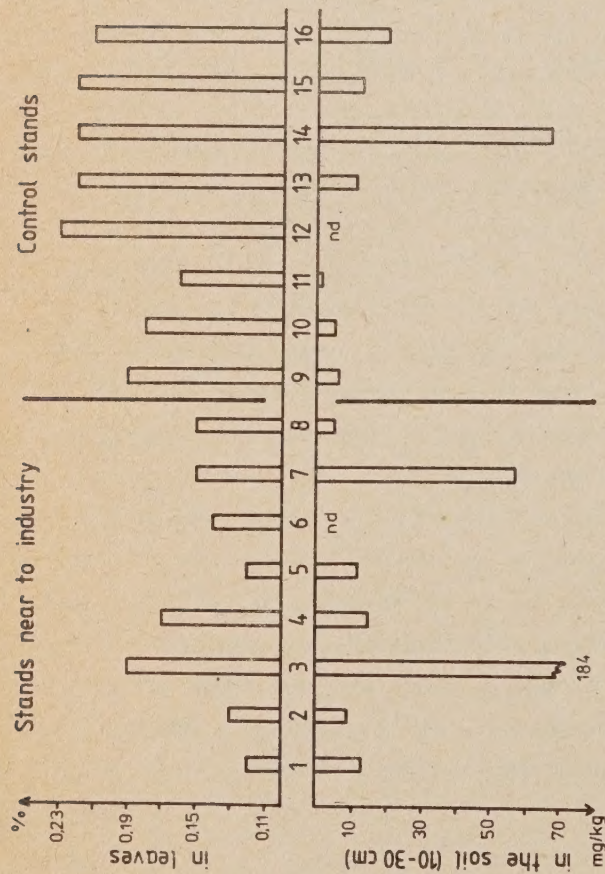


Fig. 2. P-content of the examined sessile oak stands.

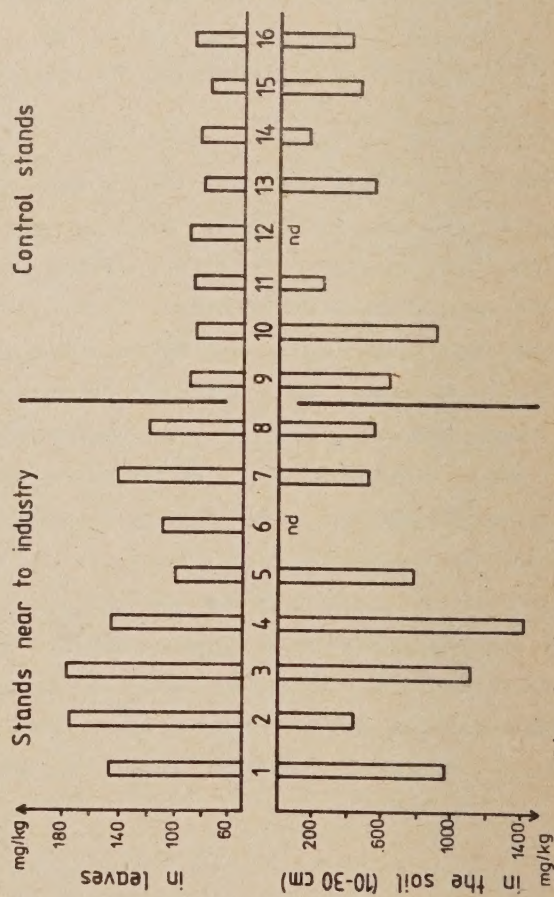


Fig. 4. Al-content of the examined sessile oak stands.

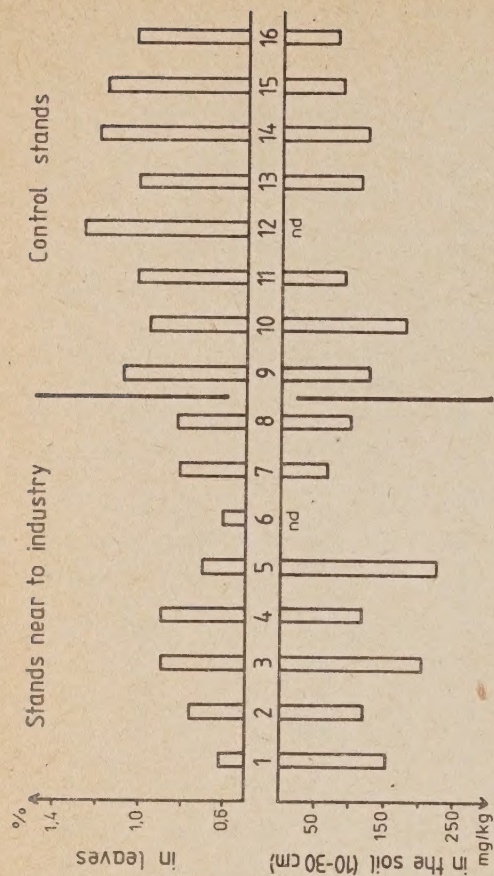


Fig. 3. K-content of the examined sessile oak stands.

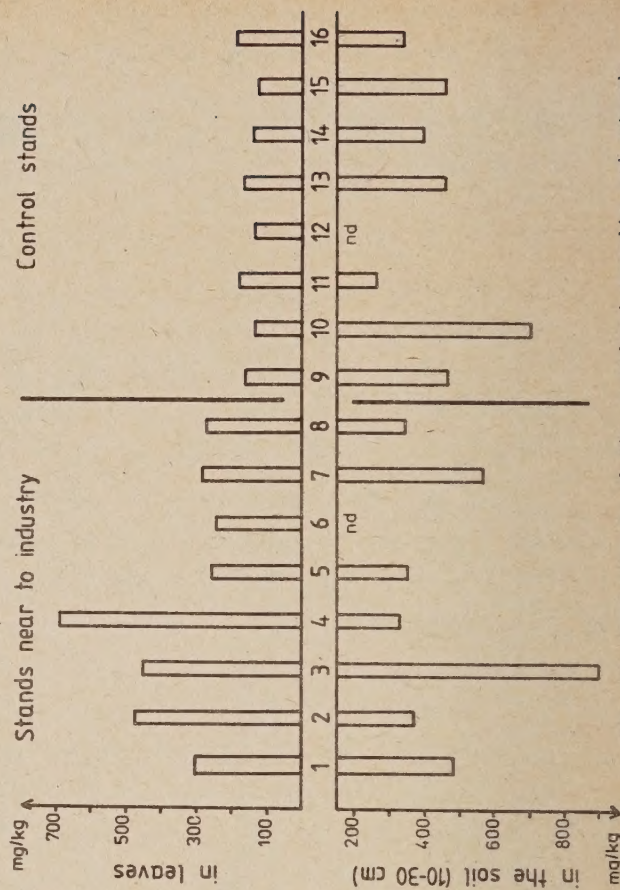


Fig. 5. Fe-content of the examined sessile oak stands.

In the leaves of the control oak stands the Ca/Al ratio is twice that of the stands near the industrial district (Tab. 2).

Leaf analyses of sessile oak stands have been performed in Hungary before by several researchers (JÁRÓ, 1962; TÖLGYESI, 1965, 1969; PAPP, 1985).

If we compare the leaf-analysis data of the 16 forests studied by us with the analyses performed 20 - 25 years ago in other sessile oak stands in Hungary (TÖLGYESI, 1965, 1969) then we can make the following establishments:

With respect to the mean Ca values of 20 years ago (9500 mg/kg) the Ca content of the leaves has decreased, whereas the Mn content has increased in all stands under study as compared to the earlier value (564 mg/kg). The P content measured 20 years ago (0.18 %) is higher than those obtained in 1987 in the leaves in the near-industry area, but somewhat lower than the mean control values. The Fe content of the leaves of the control stands is identical with the values measured two decades ago.

On comparing the results of our leaf analyses with the nutrient element limit values established by BERGMANN (1983) for oaks we can state that the P, K and Mg content of the leaves in the control stands falls within the interval given by Bergmann. The same values for the near-industry stands do no longer reach the lower limit!

The leaves of the sessile oak stands near the industrial zone contain somewhat more of the heavy metals that are normally present in the leaves only in very slight quantities (Co, Cd, Hg, Li, Pb; Table 2). This excess may be partly due to local air pollution or indirectly to the amount of soluble forms, which are increased in the acidified soil.

C O N C L U S I O N S

The conclusion of the present study is that in the soils and leaves of the diseased sessile oak stands near the Northern Hungarian Sajó-valley industrial district local industrial air pollution induces changes in element content.

As a result of air pollution the soil of the near-industry stands has become more acidic than that of the control stands. The NO_3^- and readily soluble Al content of the soils has increased.

Moving nearer the emission sources of the industrial district the S, Al, Fe and Mn content increases in the leaves, whereas the amount of several important macro-elements (P, K, Ca, Mg) decreases.

These changes in element content have considerably altered the proportion of elements in the soil and leaves of the sessile oak stands. The unfavourable changes in ecophysiological element content may play a role in the faster rate and higher degree of decay of the forests exposed to greater local industrial air pollution.

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Indication of air pollutants by means of the chemical analysis of *Robinia pseudo-acacia* leaves

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Living organisms are being more frequently used to indicate and measure air-pollutants as well as the complex impact of pollution on the environment. The Man and Biosphere Program of UNESCO among others aims at identifying biological indicators. In Hungary it is planned to set up a country-wide monitoring network in order to keep track of pollution and check the effects of different air pollutants.

Various heavy metals are present in the dust of cities and industrial areas, a great number of which have potentially carcinogenic or mutagenic effects. To indicate heavy metal presence the so called accumulation indicator organisms are mainly used.

Accumulation indicators are those plants capable of accumulating elements in their leaves after taking them up mainly by cuticular absorption without suffering visible damage. Thus leaves are the most suitable indicators. It is advisable to choose as indicators either wild or cultivated species which frequently occur and have a large areal distribution.

Robinia pseudo-acacia fulfills these requirements. This species was introduced into Hungary from America in the 1700-s. It has become widespread all over the country. *Robinia pseudoacacia* accounts for 1,517.934 hectares i. e. 18 % of total forest cover in Hungary. Owing to its adaptability it can be found in different climatic conditions and on different soils. This species is frequently found in our towns and villages and also on roadsides. *Robinia* is tolerant of air and soil pollutants (e. g. salt used to de-ice roads).

The element content of *Robinia* leaves has also been used as indicator in Western Europe.

METHODS

Heavy metals can be traced in tree-leaves in larger quantities in late August and early September. This applies to *Robinia* as well, thus these months are the recommended period for sampling. There is a basic need to have a universal sampling method for the whole surveyed area. Wagner's method (1984) has been taken into account. From a height of about 2.5 - 3 metres light leaves were chosen, approximately one kilogram from each tree. The samples were taken into the laboratory within 24-36 hours. Two consecutive washing processes were applied, each for thirty seconds: the leaves were washed free of dust and other surface depositions firstly in ordinary running water, then in distilled water. After washing they were dried in an exsiccator at a temperature of 60-80 centigrade. Following acidic exploration atomic absorption spectrophotometry was used to carry out chemical analysis. The chemical composition of *Robinia* leaves has been surveyed in Budapest and its vicinity and also in some industrial areas (e. g. Visonta, Ajka).

BOHÁČ J., RŮŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deteriorationis Regionis. Institute of Landscape Ecology CAS, České Budějovice, pp. 243-246.

Tab. 1. Chemical composition of leaves of Robinia pseudoacacia in urban (n = 40) and rural (n = 5) areas

	%						mg . kg ⁻¹				
	N	P	Ca	K	Na	Mg	Fe	Mn	Pb	Zn	Cu
Urban area (Budapest)	4.10	0.22	1.45	1.73	0.07	0.37	456	33	38	55	14
Rural area (Vácrátót)	4.72	0.23	2.05	0.87	0.06	0.30	142	76	11	23	10

THE ELEMENT CONTENT OF ROBINIA PSEUDO-ACACIA IN URBAN-INDUSTRIAL AND CONTROL AREAS

There was no significant difference in the macroelement content (N, P, Ca, K, Mg, Na) of the samples from urban (Budapest) and those of rural origin (pollution free control sites, see Tab. 1). However, major differences were found in the microelement contents of the leaves from urban-industrial and rural areas. Due to more intensive corrosion and air pollution of industrial and traffic origin, leaves in inner city areas contain larger amounts of heavy metals (e. g. Fe) than in the control areas. The highest amount of lead can be detected in places with heavy traffic (e. g. Deák-square, Keleti station, etc.).

In the vicinity of Budapest the highest amounts of the surveyed heavy metals (e. g. lead) can be found in the leaves collected in industrial areas (Vác, Dunakeszi) and in areas affected by heavy traffic (Alsónémedi).

Numerous rare elements can be found in the chemical composition of dust in the cities due to industrial production. The leaves of Robinia pseudo-acacia contain several potentially toxic elements. There is also Bi, W, Nd, Cs, I, Ag and Sc among the 33 elements identified in Budapest. A good example is a factory, where bulbs are manufactured. This site is characterised by the presence of high concentrations of tungsten and zirconium (Tab. 2). The amount of W and Zn in the leaves of Robinia pseudo-acacia and Acer platanoides collected at that site has been compared. The following results were obtained: W 58 mg . kg⁻¹, Zr 27.8 mg . kg⁻¹ and W 8 mg . kg⁻¹, Zr 2.5 mg . kg⁻¹, respectively. Both elements were contained in larger quantities in Robinia leaves.

Samples collected near to a power station fuelled with lignite (Visonta) contained 40 microelements. Amongst them Ga, Sc, In and Cd were detected (Tab. 3). When burning lignite a large quantity of sulphur is emitted. Robinia can also be used as an accumulation indicator to detect it.

DRAWING UP "EFFECT CADASTRES" BY MEANS OF BIOLOGICAL INDICATORS

Effect cadastre means the recording of air pollutants in space. The data to be registered are obtained by bioindication. The measuring stations of the monitoring network are evenly distributed. Besides conveying information about air-pollution, effect cadastres can be used to measure the degree of damage sensitive species suffer and also to obtain data about the accumulation of various elements (e. g. heavy metals) in living organisms. The division of the network and the density of measuring stations set up depends on the expected pollution loading

Tab. 2. Chemical composition of Robinia pseudo-acacia leaves (mg . kg dry matter⁻¹) in Ujpest (near to a bulb manufacturing plant) and Vácrátót (control). T - toxic, R - carcinogenic, Rs - suspect carcinogenic. + present, but unmeasurable due to overlapping, x - 0.1 - 0.2 %, xx - 0.2 - 1.0 %, xxx - 1.0 %.

Ujpest				Vácrátót			
T	Bi	4.64	-		Sr	xx	xx
T	Pb	11.60	1.21		Br	+	+
	W	58.00	-	Rs	As	+	+
	Nd	0.46	-	T	Ga	1.16	0.60
	Pr	0.11	0.12	R	Zn	40.60	21.74
	Ce	2.32	0.48	R	Cu	69.60	30.19
T	La	0.46	0.24	R	Ni	6.96	7.24
T	Ba	x	x	R	Co	0.46	0.94
	Cs	0.46	-	Rs	Fe	xxx	xxx
	I	1.97	-	Rs	Mn	x	xx
	Sb	3.48	1.81	R	Cr	9.28	2.41
R	Sn	2.90	0.60		V	4.06	1.81
T	Ag	3.48	-	Rs	Ti	x	72.46
	Mo	x	6.04		Sc	+	-
	Nb	0.11	0.12		F	2.90	1.21
T	Zr	27.84	0.60		B	x	44.68
Rs	Y	2.90	0.24				

Tab. 3. Chemical composition of flying dust of the power station and of Robinia pseudo-acacia leaves in Visonta (mg . kg dry weight⁻¹). 1 Deposited dust from the premités of the power station, 2 Robinia pseudo-acacia Visonta. ++ - 1-10 %, +++ - >10 %.

1		2		1		2		1		2	
U	2.85	-		Ce	36.64	3.2		As	8.14	-	
Th	3.26	-		La	12.21	1.40		Ge	3.66	4.7	
Bi	0.10	4.02		Ba	93.65	65		Ga	3.05	0.9	
Pb	6.72	8.8		Cs	1.20	0.038		Zn	48.86	40	
Tl	-	-		J	-	0.085		Cu	85.50	44	
W	1.00	-		Te	-	27		Ni	101.79	17	
Hf	0.90	-		Sb	0.92	0.21		Co	20.36	1.2	
Lu	0.12	-		Sn	3.76	16		Fe	+++	1300	
Yb	1.08	-		Ag	-	0.23		Mn	793.97	130	
Tn	0.20	±		In	-	0.81		Cr	264.66	4.9	
Er	1.12	-		Cd	-	0.94		V	365.37	1	
Ho	0.35	-		Mo	5.90	1.2		Ti	++	130	
Dy	2.24	-		Nd	9.16	0.19		Sc	109.93	0.23	
Tb	0.33	-		Zr	67.18	1.6		Al	244.30	1100	
Gd	2.24	-		Y	16.49	0.18		F	-	0.96	
Eu	0.71	-		Sr	75.32	150		B	-	100	
Sm	2.85	-		Rb	12.62	5.9		Be	-	-	
Nd	15.06	0.40		Br	0.30	16		Li	-	-	
Pr	4.27	0.28		Se	1.71	1.1					

and the consequent harm to the area. The squares of the network measure 1 x 1, 2 x 2, 4 x 4 km-s in areas affected by heavy loading, while in areas with clean air 16 x 16 km is regarded as enough. Each of these squares contain 20-50 measuring points.

Effect cadastre surveys have been carried out in some European countries, such as Austria, Switzerland, West-Germany (Baden-Württemberg, Saarbrücken, etc.) and Belgium. In Holland the Ministry of Health and Environmental Protection brought in a measure to build up an automatic network to record the values of SO_2 , O_3 , NO_2 and CO. Besides using measuring apparatuses biological indicators are also in use at some 40 places.

In Hungary it is also planned to set up a regular monitoring network linked with the systems of other European countries. Having identical species and standardized methods it would make possible to obtain comparable data at international level.

We recommend Robinia pseudo-acacia, as a possible indicator species for the international monitoring system. The chemical composition of its leaves indicates a good estimate of pollution loading an area. For example in Ajka and its environs polluted by a power station, an aluminium smelter and a glassworks it was possible to determine the loading rate of certain sites on the basis of data (Al, Cd, Cr, Cu, Ga, Ni, Pb, V) obtained from the leaf-analysis of Robinia.

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Plant surfaces as bioindicators of air pollution

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E I N L E I T U N G

Die Oberfläche der Assimilationsorgane stellt die Kontaktstelle und Grenzschicht zwischen der Pflanze und der Atmosphäre dar. Es stellt sich die Frage, ob mikromorphologische und/oder physikochemische Eigenschaften der Blattflächen für die Wirkungsweise von luftgetragenen Schadstoffen entscheidend sind und ob diese Eigenschaften durch den Immissionseinfluss in nennenswerter Weise verändert werden. In zahlreichen neueren Untersuchungen - vor allem an Koniferennadeln - treten die der Kutikula aufgelagerten Epikutikularwachse in den Vordergrund (Literaturübersicht bei BIERMANN, 1987). Die natürliche, altersbedingte Erosion dieser Wachse wird demnach durch die zusätzliche Luftbelastung beschleunigt. Dies führt zu einer verminderten Lebenserwartung der Nadeln (FOWLER et al., 1980; SAUTER, VOSS, 1986).

Zur Überprüfung dieser Zusammenhänge sollte die Blattoberfläche einer gut geeigneten Pflanze untersucht werden. Es sollte überprüft werden, ob die diskontinuierliche Einwirkung von Schadgasen in umweltrelevanter Konzentration zu einer qualitativen Veränderung der Wachse und zu einer Beeinflussung der Oberflächeneigenschaften führt. Zur Beurteilung wurden folgende Kriterien herangezogen:

- Ausprägung der Epikutikularwachse
- Beeinflussung der Blattbenetzbarkeit
- Veränderung des pH- Wertes und des Pufferungsvermögens der Blattoberflächen.

M A T E R I A L U N D M E T H O D E N

Pflanzenmaterial

Als Untersuchungsobjekt diente Bärlauch (*Allium ursinum*), der in der Krautschicht eines Perlgras-Buchenwaldes (*Melico-Fagetum allietosum*) als dominierende Art auftrat.

Begasungsexperimente

Am natürlichen Wuchsort wurde die Bodenvegetation des Perlgras-Buchenwaldes in Open- Top-Kammern (STEUBING et al., 1986) mit SO_2 , SO_2 und NO_2 bzw. SO_2 , NO_2 und O_3 mit umweltrelevanten Konzentrationen diskontinuierlich begast.

Begasungskonzentrationen:

$$\begin{aligned}\text{SO}_2 &: 300 \mu\text{g SO}_2/\text{m}^3 \\ \text{SO}_2/\text{NO}_2 &: 300 \mu\text{g SO}_2/\text{m}^3 + 100 \mu\text{g NO}_2/\text{m}^3 \\ \text{SO}_2/\text{NO}_2/\text{O}_3 &: 300 \mu\text{g SO}_2/\text{m}^3 + 100 \mu\text{g NO}_2/\text{m}^3 + 200 \mu\text{g O}_3/\text{m}^3\end{aligned}$$

Die Begasung erfolgte in der Vegetationsperiode von Mai bis Oktober 1986 einmal pro Woche 48 Stunden lang, wobei die Ozonbegasung nur tagsüber stattfand. Die ersten Untersuchungen wurden bereits nach der zweiten Begasungswoche (mitte Mai) durchgeführt.

BOHÁČ J., RŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deterioration of Regions. Institute of Landscape Ecology CAS, České Budějovice, pp. 247-252.

Bonitierung der Blattschäden

Als Kriterium für den Schädigungsgrad diente die Gelbspitzigkeit der Blätter. In einer 4 - stufigen Skala wurden die Blattschäden in Bonitierungsklassen folgendermassen eingestuft:

Klasse	Schädigungsgrad der Blätter
0	ohne sichtbare Schäden, 0 % der Blattfläche geschädigt
1	schwach geschädigt, 1 - 10 % der Blattfläche geschädigt
2	mittelstark geschädigt, 11 - 20 % der Blattfläche geschädigt
3	stark geschädigt, 21 - 50 % der Blattfläche geschädigt

Rasterelektronenmikroskopische Untersuchungen

Die Elektronenmikroskopischen Untersuchungen der Blattoberflächen wurden an luftgetrockneten herbarisierten Blättern mit einem JOEL- JSM- S1 durchgeführt.

Oberflächenbenetzbarkeit

In Anlehnung an die Methoden von LINSKENS (1950) und BAU (1980) wurden Blätter der Testpflanzen mit beidseitig klebendem Klebefilm auf Objektträgern horizontal ausgerichtet. Hierauf wurden mit einer Mikroliterspritze 10 μ l grosse Wassertropfen (A. dest.) appliziert und der Randwinkel, den der Tropfen mit der Luft und der Blattoberfläche bildet, mit einem Winkelgoniometer gemessen.

Oberflächen- pH- Wert

Die Bestimmung des Blattflächen- pH- Wertes wurde mit einer Mikro- pH- Einstabmesskette mit Flachmembran durchgeführt.

Neutralisierungskapazität

Mit der selben Mikro- Elektrode wurden pH- Wertveränderungen in applizierten 50 μ l- Tropfen (pH 3,5) auf der Blattoberfläche gemessen. Zur Kontrolle wurden entsprechende Tropfen auf Parafilm appliziert und deren pH- Veränderung erfasst.

E R G E B N I S S E

Allium ursinum reagierte bereits nach der zweiten Begasungsperiode mit makroskopisch sichtbaren Schädigungen auf die zugeführten Immissionen. Vor allem die Kombinationsbegasung mit Ozon führte zu einer sich rasch ausweitenden Gelbspitzigkeit der Blätter (Abb. 1).

Die REM- Untersuchungen zeigten eine zunehmende Modifizierung der Wachsstrukturen (Abb. 2). Während die Blätter der Kontrollpflanzen eine mehr oder weniger homogene Verteilung stäbchenförmiger Wachskristalle auf der Unterseite aufwiesen, verschwanden diese Strukturen bei den begasteten Pflanzen und der Bedeckungsgrad nahm stetig ab. Besonders gross erschienen die wachsfreien Flächen auf den Blättern der Pflanzen aus der Kombinationsbegasung mit SO₂, NO₂

und Ozon. Eine Quantifizierung dieser Wachsdegradation konnte bisher noch nicht zufriedenstellend erreicht werden, wird aber angestrebt.

Die Benetzung der Blattoberfläche zeigte eine Beziehung zum Schädigungsgrad der Blätter (Abb. 3). Auf den stärker geschädigten Blättern konnte eine bessere Benetzbarkeit gemessen werden. Die in Kombination mit O_3 begasten Pflanzen wiesen in allen Bonitierungsklassen die gravierendsten Veränderungen der Randwinkel auf.

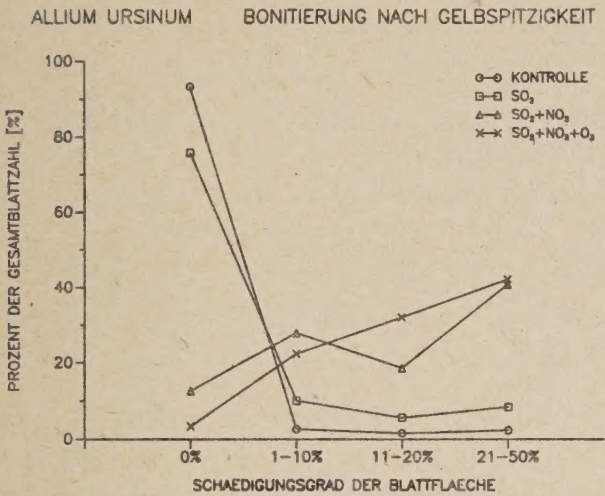


Abb. 1. Anteil der unterschiedlich stark geschädigten Blattflächen nach zweimaliger Immissionsbelastung mit SO_2 , $SO_2 + NO_2$ bzw. $SO_2 + NO_2 + O_3$.

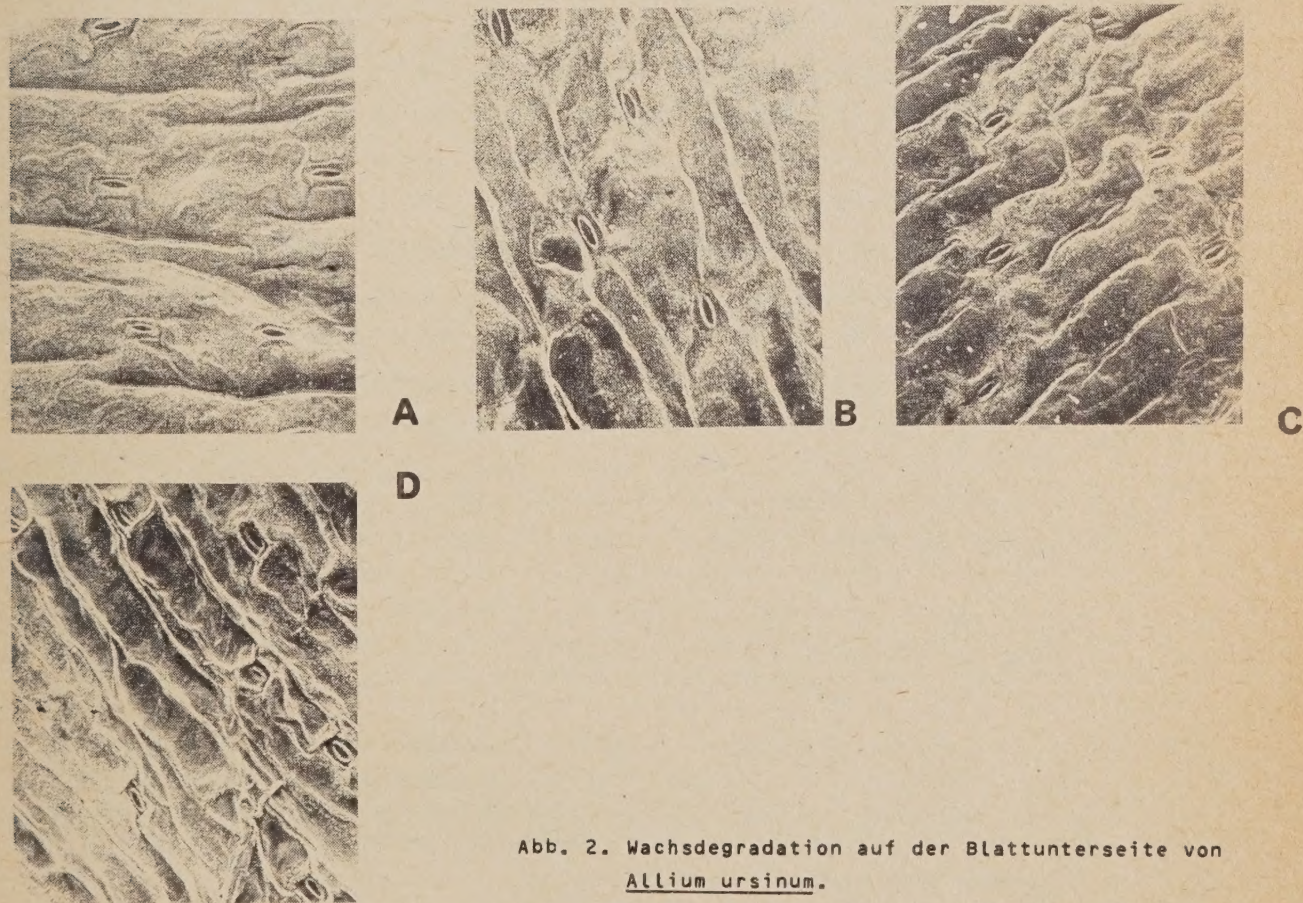


Abb. 2. Wachsdegradation auf der Blattunterseite von Allium ursinum.
A - Kontrolle, B - SO_2 , C - $SO_2 + NO_2$, D - $SO_2 + NO_2 + O_3$.

Tab. 1. pH- Werte der Blattoberflächen (adaxial) nach zweiwöchiger Begasung; Kontrollpflanzen: $5,48 \pm 0,10$.

Variante	Schädigungsgrad der Blattflächen			
	0	1	2	3
SO ₂	5,37 ± 0,14	4,76 ± 0,18	5,9 ± 0,53	5,18 ± 0,25
SO ₂ /NO ₂	5,08 ± 0,17	4,99 ± 0,26	4,86 ± 0,17	5,35 ± 0,23
SO ₂ /NO ₂ /O ₃	5,47 ± 0,12	5,39 ± 0,23	5,77 ± 0,34	5,26 ± 0,40

Tab. 2. pH- Werte der applizierten Tropfen nach 16- stündiger Verweildauer auf der Blattoberfläche von Allium ursinum (Vergleich: Parafilm - pH 3,5).

Variante	Schädigungsgrad der Blattflächen			
	0	1	2	3
Kontrolle	3,8 ± 0,1	-	-	-
SO ₂	3,8 ± 0,14	3,76 ± 0,17	4,22 ± 0,51	4,33 ± 0,56
SO ₂ /NO ₂	3,84 ± 0,20	3,95 ± 0,40	4,10 ± 0,3	4,43 ± 0,36
SO ₂ /NO ₂ /O ₃	3,98 ± 0,32	3,95 ± 0,33	4,56 ± 0,66	5,97 ± 0,58

Im Hinblick auf den pH- Wert der Blattoberflächen war nach der zweiwöchigen Begasungsdauer noch kein eindeutiger Trend zu erkennen (Tab. 1).

Nach weiteren Begasungen nahm der pH- Wert der Blattoberflächen - vor allem der mit Ozon begasten Pflanzen - deutlich ab.

Der pH- Wert der applizierten Tropfen wurde auf den unterschiedlich vorbehandelten Blättern in folgender Weise verändert (Tab. 2).

Ausgehend von einem pH von 3,5 stieg der pH- Wert der applizierten Tropfen nach ca. 16 Stunden Verweildauer auf der Blattfläche um fast 2,5 pH- Einheiten auf 5,97 an.

DISKUSSION

Allium ursinum reagiert bereits nach zweimaliger, definierten Immissionsbelastung mit deutlich sichtbaren, unspezifischen Schadsymptomen, die sich auch in den rasterelektronenmikroskopischen Untersuchungen auf mikroskopischer Ebene widerspiegeln. Unter dem Einfluss der Schadgase werden die epikutikularen Wachsschichten sowohl auf der Blattunterseite als auch auf der Oberseite

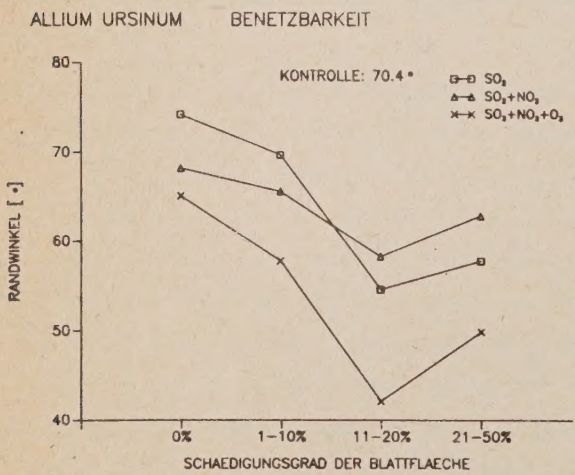


Abb. 3. Benetzbarkeit der Blattoberflächen von Allium ursinum in Abhängigkeit von der Begasungsvariante und dem Schädigungsgrad der Blätter.

in der Weise modifiziert, dass durch Wachsdegradation grossflächige Kahlstellen entstehen können. Dies wirkt sich wiederum auf die Benetzbarkeit der Blattoberfläche aus, die nicht zuletzt in hohem Masse durch die Mikromorphologie und die Verteilung der Wachsstrukturen mitbestimmt wird (JUNIPER, 1969; LINSKENS, 1950; RENTSCHLER, 1971). Durch die zunehmend lückenhafte Verteilung der Wachsstrukturen wird die Oberfläche leichter benetzbar, die Aufnahme gelöster Schadstoffe begünstigt und damit der Schädigung Vorschub geleistet.

Die verbesserte Benetzbarkeit scheint sich auch fördernd auf das Neutralisierungsvermögen der Blattoberfläche auszuwirken. Die pH- Wert- Veränderung in den applizierten Tropfen erfolgt wahrscheinlich zum Überwiegenden Teil durch den Austausch von H^+ - Ionen aus den Tropfen gegen Kationen aus dem Blatt (ADAMS, HUTCHINSON, 1984), d. h. pH- Differenzen treten besonders dann auf, wenn Ionen im hohen Mass aus dem Blatt ausgewaschen werden können. Der beachtliche pH- Anstieg in applizierten Tropfen auf Ozon- begasten Blättern lässt sich damit erklären, dass das Auswaschen (Leaching) durch Ozon- bedingte Membranzerstörungen wahrscheinlich unterstützt wird (OSSWALD, ELSTNER, 1985).

Systematische Untersuchungen zum Phänomen der Auswaschung werden zur Zeit durchgeführt.

Es sei darauf hingewiesen, dass alle Untersuchungen nur an grünen, nicht sichtbar geschädigten Blättern bzw. Blattstücken durchgeführt wurden.

Z U S A M M E N F A S S U N G

Allium ursinum wurde am natürlichen Wuchsort mit SO_2 , NO_2 und O_3 kontaminierter Luft in Open-Top- Kammern diskontinuierlich begast. SO_2 wurde allein oder im Kombination mit NO_2 bzw. in einer Kombination mit NO_2 und O_3 der Umgebungsluft zugeführt. Nach kurzer Begasungsdauer wurden die unterschiedlich behandelten Pflanzen auf die Mikromorphologie ihrer Epikutikula, die Wasserbenetzbarkeit der Blätter sowie den pH- Wert und die Neutralisierungsfähigkeit der Blattoberflächen untersucht. Die Experimente zeigten, dass die gewählten Begasungsvarianten eine Zerstörung der Wachsstrukturen, eine bessere Wasserbenetzbarkeit und eine steigende Neutralisierungsfähigkeit der Blattoberflächen verursachten. Die Ergebnisse korrelierten mit dem makroskopisch sichtbaren Schädigungsgrad der Blattflächen.

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An example of selected spruce stands reactions to air pollution

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During the period 1984-1986 regular analyses were performed on eight test areas in the Jizerské hory Mts., that differ in the degree of air pollution impact. The analyse made it possible to determine the soil reaction, the average degree of stands damage (ADSD), needles chlorophyll content, photosynthetic characteristic and annual tree-ring investigations. The objective of this studies was to state the sensitivity of individual parameters to the air pollution impact.

The Jizerské hory Mts. are a typical landscape complex which involves many important values from the points of view of natural science and landscaping. The most important ecosystems are mountain peat moors with dwarf pine, mountain beech forest, mountain detrital forest, mountain and cultivated spruce stands, mountain pastures and meadows. More than 70 % of the total area of the Jizerské hory Mts. are covered with forest stands. The original beech-fir stands have been substituted for spruce monocultures (*Picea abies* L. Karst.), they represent about 90 % of the stands at present. Under the conditions of Sudety, spruce stands form the alpine forest limit, because in the Jizerské hory Mts. there is the most unfavourable climate of the whole Central Europe for them, with regard to the altitude and extremely poor soils. The remaining 10 % is formed of deciduous trees with the prevalence of beech (*Fagus sylvatica* L.) and dwarf pine (*Pinus mugo* Turra) on peat moors (KRIX, ŠKAPEC, 1985). On deforested clearings meadows enter their position with prevailing *Calamagrostis villosa* Gmel. It forms a grassland which predominates in competition over the natural and intentional seedlings of tree species. During the last two decades the environment of the Jizerské hory Mts. was impacted by industrial air pollution. With regard to the prevailing north-west and west air flow and short distances from the sources of air pollutions, there is a high probability of the direct impact of air pollutions on the forest stands of the Jizerské hory Mts.

The investigations were carried out on the test areas established by the researchers of the State Institute of Wild-Life Conservation (SÚPPOP), Prague in 1980 for the needs of the territorial research of the damages to spruce stands. The research was started on 7 areas in 1984. In 1985 the test area Hřebínek was involved. It represents a well preserved spruce stand at higher altitudes of the Jizerské hory Mts. The test areas were determined in such a way to include spruce stands: at different altitudes, of different age, on different expositions, on different slopes of the terrain, on different typological units, at different zone of exposure, at different zone of exposure, at different stage of injury. On test areas a sample tree was selected for ecophysiological research. The sample tree was selected in such a way so that its degree of tree damage might correspond to the average degree of stand damage of the given test area. This sample tree was used for determination of photosynthetic characteristic and determination of chlorophyll content. Within the scope of each area the average degree of stand damage (ADSD) was determined according to the methods of SÚPPOP Prague (KRIX, ŠKAPEC, 1983). Determination of the chlorophyll content in acetone extract was performed spectrophotometrically. The measurement of the photosynthetic characteristic of spruce needle was based on gasometric methods. The measure-

ments were carried out with use of a portable photosynthesis system LI-6000 (LI-COR, USA). The net photosynthesis rate and stomatal resistance to CO_2 transfer was measured in forest on test areas. The measurements were carried out on intact shoots, without separation of twigs from tree. Therefore, during the vegetative period of 1984 changes in active and exchange soil reactions were determined, as a complex indicator of soil condition. The experiments were performed on test areas of the Jizerské hory Mts. On each area the sample of soil was taken from the upper part of the soil horizon B in three accidentally selected spots. At the end of the growing season of 1986 and 1987 material for dendrochronological studies was taken. The sampling (cores) were obtained from adult trees on the given test areas. Two opposite cores were taken breast height $d_{1,3}$ of 10 sample trees. The sample trees were selected using the method of the mean stem. In the case of large destruction of the stand under the impact of air pollution the number of sample trees was reduced, up to the minimum of 6 (i.e. 12 cores). All cores were measured in the laboratory using an annual-ring width measuring apparatus. The width of individual annual ring was measured to the nearest tenth of a mm.

The test areas are divided into three groups. The areas with the ADSD under the value of 3 are Hřebínek and Cikaňák. The ADSD value under 4 was obtained for the areas Nová Louka, Bukovec and Václavikova studánka. The areas Prales Jizera, Černá hora and Černá jezírka have the ADSD value higher than 4.5. The changes in ADSD values of test areas during the period from 1984 to 1986 are demonstrated. The increase of ADSD was obtained for all test areas. This increase well documented the chronic injury of forests in the Jizerské hory Mts. From the analysis of chlorophyll content over the period from 1984 to 1986 differences were obtained between individual test areas. The individual groups of test areas (partition according to the values of ADSD) is possible to identify from the results of pigment analysis. The chlorophyll content of needles is responsible for monitoring of air pollutants impact. The measurements of needles photosynthetic characteristics were carried out in September 1985 (age class of needles 1985). In this period the needles were physiologically fully matured (KOCK, 1976). Each test area was evaluated on the base of 90 measurements of photosynthetic characteristics. The values of net photosynthetic rate of spruce are able to distinguish individual test areas. The difference between the test area Hřebínek (the best state of health and Prales Jizera (the worst state of health) amounts to $8.17 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. There are significant differences (significance level of 95 %) between individual groups of test areas. The values of CO_2 compensation concentration confirm a different possibilities of photosynthetic activity of spruces from individual test areas. The value of CO_2 compensation concentration has a significant biomonitoring ability. An interesting information about relationships between air pollution impacts and photochemical reactions of spruce needles photosynthetic apparatus is possible to obtain from the values of the compensation photon fluence rate and photosynthetic efficiency. The value of the compensation photon fluence rate provides an information about an equilibrium between processes of assimilation and dissimilation in relation to radiation energy. Test areas with a stronger impact of air pollutants have higher values of the compensation photon fluence rate. This documented worse conditions of the photochemical apparatus of needles from this test areas. The values of photosynthetic efficiency give important information about costs of energy for photosynthesis. The photosynthetic apparatus of needles from the test

area Prales Jizera requires 2.42 times higher input of sun radiation in comparison with area Hřebínek. From these results follows, that stands under higher impacts of air pollution have significantly worse possibilities to energy building, assimilates production and survival. The stomatal resistance to CO_2 transfer forms a very important defensive mechanism of plant to penetration of nearly all types of pollutants. There are significant differences (significance level of 95 %) between values of stomatal resistance to CO_2 transfer from the test area Černá hora, Prales Jizera and the others areas. The value of the stomatal resistance to CO_2 transfer of needles from the test area Černá hora is 4.3 times higher than one from the test area Cikaňák. Thus in this case the stomatal resistance have a very good distinguish ability for monitoring of air pollution. The soils of the Jizerské hory Mts. have strong or very strong acid reaction. The high soil acidity is caused by the type of parent rock and during the last decades it was affected by air pollution. When comparing the values of active soil reaction obtained on the test areas in 1984 with the data by Pelišek (1968) it can be stated that during 16 years an active soil reaction decrease by 0.8 pH on the average was recorded in the B-soil horizon of the forest soils of the Jizerské hory Mts. In the growing season, fluctuation of soil reaction values was exhibited on all areas. It is caused by the course of forest stand nourishment, humification changes, by the course of microbiological processes and, particularly, by weather changes. The soils of the zone of humus podzols characterized by the altitude of more than 900 m a.s.l. (the test areas Prales Jizera and Černá hora) have lower soil reaction than those of the lower zone of brown forest soils (the test areas Bukovec, Nová louka, Václavikova studánka and Cikaňák). The results correspond to the data by Pelišek (1986) who reported, that the soil acidity increased to the soil zones of mountain altitudes. For comparison of individual test areas on the basis of tree-ring analyses not very homogenous material was available. The determined age of sample trees indicated that in 1986 the age of spruce stands ranged from 53 to 148 years on the test areas Hřebínek and Černá jezírka, respectively. The youngest stand (the test area Hřebínek) differed most by its physiological age phase (ASMANN, 1968). It exhibited considerably steep curve of the diameter increment. Extrapolation of increment on the basis of the previous trend was rather discutable. Within the scope of individual test areas the stands were evaluated as even-aged ones. The decrease in increment is conspicuous on all the test areas under study. Considerable decrease in increment was exhibited in 1980 after unfavourable climatic conditions at the beginning of the year. After this year the stands on the test areas Černá jezírka, Václavikova studánka and Černá hora remained in considerable increment depression due to the total weakening of the stand by air pollution. The loss in wood increment of different values has been observed on all test areas since 1980.

The relations between soil reaction and ADSD are not significant. But, the relationship between the elevation of test areas and ADSD is significant (significance level of 95 %). The soil reaction have not any relations to needles photosynthesis rate and needles chlorophyll content. The relationship between ADSD and needles photosynthetic rate has the high statistical significance (significance level of 99 %). These results confirm the very tight relation between production processes and tree environment. The relation between ADSD and chlorophyll content of needles was significant (95 %) during the period from 1984 to 1986. This result indicates the significant influence of tree environment on the pigment formation in the needles.

The utilization of the photosynthetic characteristic of spruce needles appears as to be a prospective tool of biomonitoring systems. By observation of production processes, their mechanism and relations to environment, an important question arises about the relationships between production potential (photosynthesis) and utilization of this potential in a wood formation. The wood formation is interesting from the production point of view. Significant relationship (significance level of 95 %) was found between the rate of photosynthesis and the average annual increment of spruce tree during the period from 1984 to 1986. The relation between the rate of photosynthesis and the loss of wood mass production in the year 1985 is highly statistically significant (99 %).

The results indicate that the ecophysiological research of spruce is a very powerful tool of a biomonitoring system. The physiological state of spruce stands very good corresponds with impact of the environment where these forest stands are situated.

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The influence of Ostrava's industrial agglomeration on the quality and toxicity of the Ostravice and Odra in Czechoslovakia

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Czechoslovakia is a country with relatively high density of population and high density of industrial plants too. Such concentrated activities bring about problems with affecting surrounding ecosystems. The biggest environmental problems occur in the agglomerations where the concentration of both industry and population are extremely high. Ostrava's industrial agglomeration is one of the biggest agglomerations in Czechoslovakia. There are many types of industrial technologies there including those of heavy industry, which affect wide surrounding of the town. Serious environmental problems are caused not only by wastes escaping into the atmosphere, but by great amount of wastewaters produced by the agglomeration too. The change of the character of surface water that becomes during its flow through the agglomeration was the subject of this contribution.

The area Ostrava's industrial agglomeration is situated as shown in Fig. 1. Fig. 2 shows the system of rivers flowing through Ostrava. This system involves the Odra - the most important river of Northern Moravia that has its origin in Oderské vrchy, the Opava that brings water from Jeseníky mountains and the Ostravice river that springs in Beskydy mountains.

The sampling places were situated at described rivers in these numbers: the Odra - 3 places, the Ostravice - 3 places and the Opava 2 places. The sampling places were located to characterise the changes of water quality during its flow through the agglomeration.

Loc. 1, 4 and 7 characterised water incoming to Ostrava's industrial agglomeration.

Loc. 3 was situated at the outflow from Ostrava's industrial agglomeration after Odra's receiving all wastewaters.

Loc. 6 and 8 were situated at the mouth of the rivers Ostravice and Opava respectively.

Loc. 2 and 5 were chosen to give more detailed information of gradual changes that become during the flow of the Odra and the Ostravice. Loc. 2 was situated upstream the mouth of the Ostravice, loc. 5 lied upstream the place the Ostravice receives wastewaters of Celpak paper mills - the most important source of wastewaters.

The rivers at each sampling place had not their natural characters, the river beds were canalised.

The samples were taken monthly during 1986 y. Basic physical and chemical characteristic of water was analysed using standard methods comprised in ČSN 830530 (14 parameters). Trophical potential of water was measured by cultivation method of *Scenedesmus quadricauda* under standard conditions using the method by ŽÁKOVÁ (1981), where a classification of eutrophication of water is stated too. To ensure, whether the polluted surface water has toxic effect to bacteria or not toxicity test on the Microtox system were made. The toxicity tests were made under standard conditions, e. g. 15° C and 15 minutes duration of the test. Bioluminescent bacteria *Photobacterium phosphoreum* was used as testing organism. T - effect was used for evaluation of the toxicity tests. It may be expressed as

$$T = \frac{\Delta \%}{100 - \Delta \%}$$

where $\Delta\%$ is a percentual light loss of the bacterial culture caused by a sample in time (t) and temperature (T).

RESULTS AND DISCUSSION

The effect of Ostrava's industrial agglomeration on chemical composition of water is remarkable. The hydrochemical analyses comprised 14 parameters, Tab. 1 shows the medians of the most important of them.

The values of dissolved oxygen (DO) in the Odra (loc. 1, 2 and 3) gradually decrease downstream the river. Similar trend was observed in the other rivers. The values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) are oppositely growing up during the longitudinal sections of the rivers. All the three factors indicate that the rivers incomming into the Ostrava's industrial agglomeration are of quite sufficient quality and that the quality of their water is worsened during their flow through the agglomeration.

Water conductivity which is a factor comprising the influence of all dissolved ionic compounds in the water changes most of the investigated rivers at longitudinal section of the Ostravice river. At the other rivers such remarkable changes were not observed. Loc. 3 (outflow from the agglomeration) where high conductivity values were gained too is affected by the water from the Ostravice river. Almost the same situation can be observed in the chloride concentrations.

The concentrations of Fe_{tot} were usually found near $0.2 \text{ mg} \cdot \text{l}^{-1}$, at the outflow from the agglomeration was not found a significant increase. Remarkable increase however occurred down the flow of the Ostravice river (loc. 4, 5 and 6), the locality of the highest altitude of which showed the lowest concentration Fe_{tot} values from all the localities.

The trophical potential of the localities (Fig. 3) shows the water in the Odra river coming to Ostrava's industrial agglomeration is mesotrophic. Up to the mouth of the Ostravice



Fig. 1. Localization of Ostrava's industrial agglomeration in Czechoslovakia.

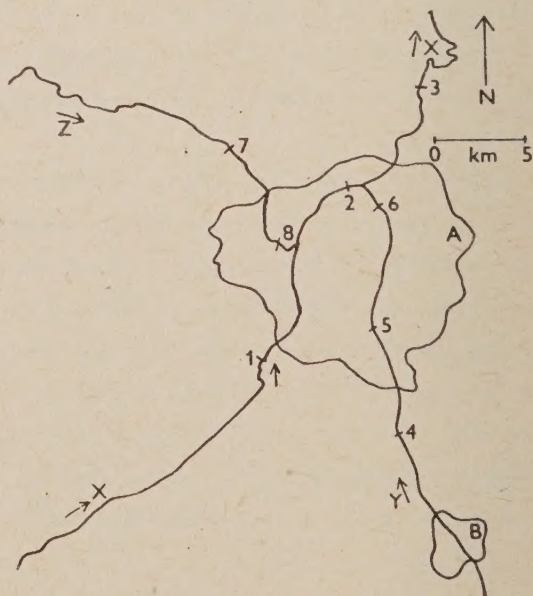


Fig. 2. Rivers in Ostrava and sampling places. 1-8 - sampling places; A, B - towns Ostrava, Frydek Mistek; X, Y, Z - rivers: the Odra, the Ostravice, the Opava.

Tab. 1. The hydrochemical analyses of surface waters (median values).

Locality	DO (mg . l ⁻¹)	COD	BOD	Cond. (μS.cm ⁻¹)	Cl ⁻	Fe _{tot.}
1 - Polanka	10.6	6.2	4.1	444.5	15.2	0.27
2 - Č. potok	9.9	8.4	6.6	441.0	19.4	0.24
3 - Bohumín	6.2	13.1	10.8	638.5	25.4	0.22
4 - Lískovec	10.7	5.7	3.2	160.0	13.4	0.06
5 - Vratimov	11.4	7.8	4.7	250.0	22.3	0.22
6 - Muglinov	6.2	39.2	32.4	690.0	157.3	0.59
7 - Jasénky	10.2	7.0	6.3	319.5	17.3	0.24
8 - Třebovice	8.3	6.9	5.7	332.5	16.7	0.19

to the Odra (loc. 2) the trophical potential remains at similar value. The median trophical potential gained at loc. 3 - the outflow from the agglomeration - is of lower values, these data are however affected by industrial wastes from Vítězný únor plant, that causes a decrease of the growth of the testing organism. The inflow of the Opava with median trophical potential of 105 mg dry weight/l at loc. 8 does not increase the trophical potential of the Odra.

The median values of the trophical potential in the flow of the Opava show slight increase between loc. 7 and 8 within the range of mesotrophy. The Ostravice shows remarkable increase of the trophical potential at localities downstream the loc. 4 - Lískovec. The loc. 5 - Vratimov is affected mainly by the outflow from the Sviadnov wastewater treatment plant that treats water from town of Frýdek-Místek. The loc. 6 - Miglinov located upstream the mouth of the Ostravice is the only locality that is classified as eutrophic. The water there is affected by wastewaters from the most of Ostrava's industrial plants the most significant of which are the Celpak paper mills, the Vítkovice steel works and the water treatment plant of the municipal wastewaters in Muglinov. As the Ostravice's water (loc. 6) is eutrophicated much more than the Odra's water (loc. 2) there should be gained an increase of the trophical potential between the loc. 2 and 3 lying on the Odra's flow. He actual situation proves the inhibition effect of the Odra's water at loc. 3 to the growth of used testing organism.

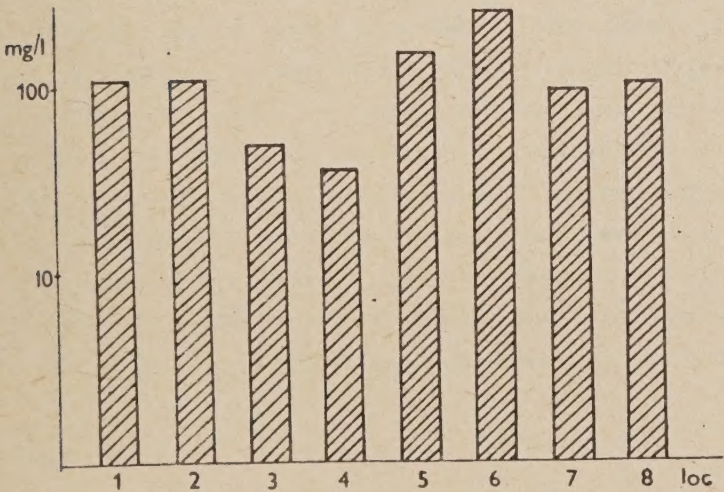


Fig. 3. Trophical potential in Ostrava's rivers (median values in mg.l⁻¹ dry weight).

Tab. 2. Toxicological evaluation of surface waters in Ostrava (Microtox 15 min, 15° C).
+ - stimulation effect observed

Locality	max effect (%)	EC 10	EC 50
		% of sample	
1 - Polanka	11.5	39.6	110.4
2 - Č. potok	-	-	-
3 - Bohumin	24.8	32.6	52.1
4 - Lískovec	3.5	+	+
5 - Vratimov	17.0	30.1	96.5
6 - Muglinov	15.2	30.6	75.4
7 - Jasénky	5.2	+	+
8 - Třebovice	7.7	+	+

In order to make the evaluation the rate of toxic effect of the river water to organisms toxicity tests were carried out using the system of Microtox. An average toxic effect actually measured after 15 min of testing time (Tab. 2, column 1) shows that the waters incomming in the Ostravice (loc. 4) and the Opava up to its mouth (loc. 7, 8) caused a maximum measured toxic effect less than 10 % of light loss of luminiscent bacteria used as test organism. The water incomming to Ostrava's industrial agglomeration in the Odra showed maximum median effect 11.5 % light loss. The highest toxic effect was noticed in the water outflowing from the agglomeration. This fact also explains why the values of the trophical potential gained at this locality were lower than it was expected. An interesting course of the toxic effect downstream the Ostravice river - loc. 5 and 6 is caused by the outflow of only partly treated water from the water treatment plant with the outflow upstream the loc. 5. As all the other wastewaters the Ostravice receipts downstream up to its mouth are biologically treated the toxic effect of the Ostravice's water is gradually getting lower. The columns 2 and 3 contain calculated concentrations that cause 10 and 50 % toxic effect, respectively. The cases marked by crosses showed stimulation effect in lower concentrations of the samples to testing organism and the calculation was thus impossible.

The localities situated on the rivers round the Ostrava's industrial agglomeration may be characterised to be affected during the flow through the agglomeration as in hydrochemical as in eutrophication as in toxical point of view.

The Ostravice shows the biggest gradient of all the factors in its longitudinal section. The water at the upstream locality - loc. 4 (Fig. 2) is evaluated to be the clearest of all, the water quality at loc. 6 is however the worst among all searched places. The eutrophication influence caused by the Ostravice's inflow to the Odra was not proved though the trophical potential of the localities 2 and 6 was relatively high. The reason was revealed by toxicity tests that showed the toxicity of river water remarkably increased downstream the loc. 2 and 6.

The results proved remarkable changes that become in the river water during its flow through the Ostrava's industrial agglomeration. Such deterioration of water ecosystems is caused by unproperly great amount of wastewaters the rivers accept from various plants. It is sure that greater budget spent on ecological investment would much improve the water quality and would decrease the water ecosystems deterioration the agglomeration shows at present.

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A comparison between artificial and natural substrates for estimation water quality indices from diatom communities analysis

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I N T R O D U C T I O N

As reviewed by COLLINS et WEBER (1978) and LOWE et GALE (1980) the algal component of the periphyton, i. e. phycoperiphyton, has been employed extensively in lotic water quality research, and more peculiarly the Bacillariophyceae (diatoms) which can be found at any season on any aquatic substrate and which can rather more easily than some other groups of algae be identified to the species level.

In this kind of research the diatom communities are sampled from their natural substrates (rocks, macrophytes, logs, ...) or from a diversity of artificial surfaces (glass or Perspex slides, clay tiles, slates, aluminium sheets, ...) which are used in order to obtain samples more uniform and easier to collect.

Mainly in respect to river organic pollution some diatom indices have been proposed in order to quantify the level of a pollution; they take into account the structure of the diatom community and either the particular species sensitivity to the content of putrescible organic matter in water (saprobic valence) either their global sensitivity. Among the most recently improved indices of lotic water quality may be mentioned the diatom indices of DESCY (1979), MOUT-HON et COSTE (1984), SLÁDEČEK (1986), FABRI (1987) and LECLERCQ et MAQUET (1987).

But it has been shown that diatom communities may differ from each other within the same water and at the same time related to the kind of their substrate (e. g. FOERSTER, SCHLICHTING, 1965) and primarily that artificial substrates bear algae communities qualitatively and quantitatively different from communities colonizing natural substrates (TIPPETT, 1970; SIVER, 1977; TUCHMAN, STEVENSON, 1980; ANTOINE, BENSON-EVANS, 1986).

The question may thus arise if the calculation of a diatom index must be based on community analysis from a particular substrate, - epilithic communities are commonly sampled -, and if the use of artificial substrates, often said a non valid ecological method, may give good results in this respect.

The object of the study presented here is to measure, with data collected in a previous investigation (not published), the impact of the sampling and of the sampled substrate on some diatom indices and more especially to test the validity of using artificial surfaces in estimating water quality with this kind of indices.

M A T E R I A L A N D M E T H O D S

The investigation has been carried on during spring 1984 in the upper Lomme, a belgian Ardenne stream. The sampling site lie at 370 m altitude, 8 km away from the spring. The flow is there rather fast and the bottom is stony, the stones being locally covered with tufts of macrophytes

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(mainly the Rhodophyte Lemanea fluviatilis and the Bryophytes Scapania undulata, Chiloscyphus polyanthus and Fontinalis squamosa). At the sampling time, the water was slightly acid (pH = 6.8), poor in calcium carbonate ($\text{Ca}^{++} = 5 \text{ mg/l}$), weakly mineralized ($\text{K}_{10}^{\text{OC}} = 54 \text{ } \mu\text{S/cm}$), well aerated ($\% \text{O}_2 = 95 - 100$) and a little bit eutrophicated owing to the drainage of manured meadows up-stream.

Microscope glass slides were used as artificial substrate in order to compare the diatom communities growing on them with the epilithic and epiphytic periphyton. Ten pairs of slides were fixed, in a vertical position and in a parallel direction to the flow, at a concrete block lowered to the stream bottom for a colonizing period of three weeks (from 4th to 26th April). The April 26th were collected at the same time and from the same stream site : the 10 pairs of glass slides, 10 samples of periphyton brushed from 10 heavy stones (vertical faces parallel to the flow) and 5 tufts of each of the three commonest macrophytes (Scapania, Lemanea and Fontinalis).

The diatom community structure was worked out by microscopical observation of about 500 frustules, identifying them to the species or variety level.

The following diatom indices (DI) were reckoned : DI_F (FABRI, 1987), DI_D (DESCY, 1979), DI_{MC} (MOUTHON, COSTE, 1984), DI_S (SLÁDEČEK, 1986) and DI_{LM} (LECLERCQ, MAQUET, 1987).

In its formula DI_F looks like the saprobic index introduced by PANTLE et BUCK (1955):

$$\text{DI}_F = \frac{\sum_{i=1}^n A_x \cdot g_x}{\sum_{i=1}^n A_x} \cdot \frac{5}{6}$$

where: A_x is the relative abundance of taxon x

g_x is a taxon sensitivity value (from 1 for resistant taxa to 6 for sensitive ones)

according to a distribution of the taxa in 6 sensitivity groups towards organic pollution.

DI_F varies from 1 (polluted waters) to 5 (natural oligotrophic waters).

The four other indices derive from the ZELINKA et MARVAN (1961) index which introduced a weighting factor related to the ecological amplitude of the organisms; the stenoeccious taxa are given a higher indicative weight. They are calculated with the following general formula:

$$\text{DI} = \frac{\sum_{i=1}^n A_x \cdot s_x \cdot i_x}{\sum_{i=1}^n A_x \cdot i_x}$$

where A_x is the relative abundance of taxon x, s_x is the sensitivity value of x and i_x its indicative weight. They differ possibly from one another in the scale of s- and i-values (see original papers for origin and accurate meaning of s-values):

in DI_D and DI_{MC} : - s-scale: 1 (resistant taxa) to 5 (sensitive taxa)

- i-scale: 1 to 3

- DI-scale: 1 (polluted waters) to 5 (unpolluted waters),

in DI_S : - s-scale: 0 (saproxenic taxa) to 4 (saprobiontic taxa)

- i-scale: 1 to 5

- DI-scale: 0 (xenosaprobic waters) to 4 (polysaprobic waters),

in DI_{LM} : - s-scale: 1 (saprobiontic taxa) to 5 (saproxenic taxa)

- i-scale: 1 to 5

- DI-scale: 1 (polysaprobic waters) to 5 (xenosaprobic waters).

Water pollution level is estimated according to the DI-classes shown in Tab. 1.

Tab. 1. DI-classes and water pollution level

	Pollution level				
	absent	weak	moderate	severe	very severe
DI_S	0 - 0.9	1.0 - 1.4	1.5 - 2.4	2.5 - 3.4	3.5 - 4.0
DI_D and DI_{MC}	5.0 - 4.6	4.5 - 4.0	3.9 - 3.0	2.9 - 2.0	1.9 - 1.0
DI_{LM}	5.0 - 4.3	4.2 - 3.6	3.5 - 3.0	2.9 - 2.3	2.2 - 1.0

RESULTS AND COMMENTS

Analysis of the 34 periphyton samples indicate some differences between the diatom communities colonizing the various substrates. They differ from one another in species composition but principally in the relative abundances of the main shared taxa. Tab. 2 displays the mean community structure per substrate; are taken into account the taxa whose relative abundance is $\geq 1\%$ in at least one substrate; most characteristic figures are underscored.

Tab. 2. Mean relative abundance (%) of the main taxa per substrate

	Substrate				
	Slides	Stones	Scapania	Lemanea	Fontinalis
<i>Fragilaria vaucheriae</i>	26.6	37.4	24.8	24.7	24.0
<i>Meridion circulare</i>	27.1	13.8	17.6	16.5	8.7
<i>Diatoma hiemale</i> var. <i>mesodon</i>	21.1	6.0	12.5	22.4	10.9
<i>Gomphonema angustatum</i> var. <i>productum</i>	5.8	4.0	6.8	6.3	7.5
<i>Cymbella ventricosa</i>	0.9	8.1	4.7	3.4	4.1
<i>Surirella ovata</i>	4.1	3.5	2.2	2.2	3.0
<i>Achnanthes minutissima</i>	2.1	6.8	4.4	2.9	6.9
<i>Nitzschia palea</i>	3.4	2.6	2.8	1.5	7.9
<i>Gomphonema parvulum</i>	2.6	0.9	1.6	0.8	2.1
<i>Fragilaria capucina</i>	0.6	1.4	2.4	2.5	2.4
<i>Eunotia pectinalis</i>	0.6	1.3	3.2	3.0	1.0
<i>Navicula minima</i>	0.7	2.8	3.9	1.6	0.8
<i>Meridion circulare</i> var. <i>constrictum</i>	0.9	1.6	2.2	3.9	1.6
<i>Fragilaria construens</i>	0.1	1.1	1.4	0.5	0.3
<i>Nitzschia archibaldii</i>	0.4	0.2	0.2	0.3	1.3
<i>Navicula saprophila</i>	0.7	1.5	0.8	0.1	0.5

The width of the disparity between substrates may be estimated by calculating a percent similarity according to the formula (LOWE et GALE, 1980:

$$SIMI = 100 - 0.5 \sum |x_i - x_j|$$

where x_i and x_j are the percentage (relative abundance) of the taxon x on substrates i and j . In Tab. 3 percent similarity is given for all the combinations between the various substrates and considering all the taxa which have a relative abundance $\geq 0.5\%$ in at least one substrate; similarity was also reckoned after averaging the communities from the three plant substrates (macrophytes).

Tab. 3. Percent similarity between diatom communities on the various substrates.

	Slides	Stones	Scapania	Lemanea	Fontinalis
Slides	-	67	81		
Stones	67	-	80		
Scapania	79	79	-	88	84
Lemanea	84	73	88	-	78
Fontinalis	74	77	84	78	-

The diatom communities on glass slides and on stones are the least similar; the macrophytes lie between the former and the latter; they bear indeed communities which are equally similar to those found on slides or on stones.

A reciprocal averaging (correspondence analysis) has besides pointed out a fairly high variability between samples from the same substrate. Stones appeared the least homogeneous substrate in spite of special care in sampling (even one sample has to be rejected as abnormal datum); on the other hand glass slides proved the most homogeneous.

The various diatom indices (DI) were reckoned for each sample and averaged per substrate. Tab. 4 displays the mean DI-values ($\bar{m}$) with their standard deviation (s) for the n samples per substrate and also, at the bottom line, the general mean. The "participation rate" of the taxa in the indices, i. e. the cumulative proportion of the taxa taken into account, was generally higher than 95 %; that means that each taxon whose relative abundance is $\geq 0.5\%$ in at least one sample per substrate is characterized by a sensitivity value for each DI and participates to the DI-values.

Tab. 4. Mean DI-values per substrate and general means

Substrate	n	DI _F	DI _D	DI _{MC}	DI _S	DI _{LM}
		$\bar{m} \pm s$	$\bar{m} \pm s$	$\bar{m} \pm s$	$\bar{m} \pm s$	$\bar{m} \pm s$
Fontinalis (F)	5	3.4 $\pm$ 0.18	3.9 $\pm$ 0.28	3.6 $\pm$ 0.31	1.3 $\pm$ 0.21	3.5 $\pm$ 0.18
Stones	9	3.5 $\pm$ 0.10	4.3 $\pm$ 0.16	4.0 $\pm$ 0.27	1.2 $\pm$ 0.17	3.6 $\pm$ 0.12
Macrophytes (S + L + F)	15	3.6 $\pm$ 0.19	4.3 $\pm$ 0.32	4.0 $\pm$ 0.35	1.1 $\pm$ 0.23	3.7 $\pm$ 0.20
Scapania (S)	5	3.6 $\pm$ 0.08	4.4 $\pm$ 0.11	1.1 $\pm$ 0.15	1.1 $\pm$ 0.15	3.8 $\pm$ 0.10
Slides	10	3.7 $\pm$ 0.08	4.5 $\pm$ 0.11	4.2 $\pm$ 0.14	0.9 $\pm$ 0.07	3.8 $\pm$ 0.11
Lemanea (L.)	5	3.8 $\pm$ 0.07	4.6 $\pm$ 0.05	4.3 $\pm$ 0.06	0.9 $\pm$ 0.11	3.9 $\pm$ 0.05
All	34	3.6 $\pm$ 0.14	4.3 $\pm$ 0.21	4.1 $\pm$ 0.30	1.1 $\pm$ 0.19	3.7 $\pm$ 0.16

Tab. 5. DI-value ranges per substrate and for all samples

Substrate	n	DI _F	DI _D	DI _{MC}	DI _S	DI _{LM}
Fontinalis (F)	5	3.3 - 3.7	3.6 - 4.4	3.3 - 4.1	1.0 - 1.5	3.3 - 3.8
Stones	9	3.4 - 3.7	4.1 - 4.6	3.5 - 4.3	1.0 - 1.4	3.5 - 3.8
Macrophytes (S + L + F)	15	3.3 - 3.9	3.6 - 4.6	3.3 - 4.4	0.8 - 1.5	3.3 - 4.0
Scapania (S)	5	3.5 - 3.7	4.2 - 4.5	3.9 - 4.2	0.9 - 1.3	3.6 - 3.9
Slides	10	3.6 - 3.8	4.3 - 4.6	4.0 - 4.5	0.8 - 1.0	3.5 - 3.9
Lemanea (L)	5	3.8 - 3.9	4.5 - 4.6	4.3 - 4.4	0.8 - 1.1	3.8 - 4.0
All	34	3.3 - 3.9	3.6 - 4.6	3.3 - 4.5	0.8 - 1.5	3.3 - 4.0

Some of the indices (Tab. 4, bottom line) may look like more pessimistic than others; they however have to be read, in evaluating the level of pollution, according to the classes presented in Tab. 1; in this respect the various mean DI-values characterize a level of weak pollution and are thus here fairly equivalent.

If attention is paid to the diversity of DI-values related to substrates it can first be pointed out that when sorted out by increasing figures (for DI_F, DI_D, DI_{MC} and DI_{LM}) or decreasing figures (for DI_S) substrates occupy the same relative position for every index.

In other respects, some substrates give here systematically lower DI-values (e. g. Fontinalis and stones) and some others higher values (e. g. glass slides and Lemanea). It's easily explained with Tab. 2, where it can be seen that slides and Lemanea are colonized by a higher proportion of sensitive taxa (Meridion circulare, Diatoma hiemale var. mesodon) whereas stones and Fontinalis bear in higher abundance tolerant (Fragilaria vaucheriae, Cymbella ventricosa) or even resistant taxa (Nitzschia palea).

DI-value differences may appear meaningless. It must be first specified that some differences as small as 0.2 or 0.3 prove however statistically significant. Student t-test point out that difference between slides and stones is highly significant ($P < 0.01$) for DI_F, DI_D or DI_S and significant ($P < 0.05$) for DI_{MC} or DI_{LM}. Macrophytes taken as a whole don't differ from glass slides except for DI_S (significant difference).

But these differences, although small, may have some importance when water quality is inferred from the diatom indices. Comparing Tab. 4 with Tab. 1 shows indeed that, according to the sampled substrate, DI_S diagnose in the Lomme sometimes no pollution sometimes a weak pollution level, DI_{MC} and DI_{LM} a weak or a moderate pollution and even that from DI_D-values pollution of this stream may be said absent (with Lemanea samples) or weak (with slides, Scapania and stones samples) or moderate (with Fontinalis samples). This lack of precision is still more striking when the whole DI-value ranges are considered (Tab. 5), and when it is kept in mind that usually diatom index is calculated from a single periphyton sample!

Setting aside the DI_F, which for pollution inference has to be related to typological reference values, DI_{LM} and DI_{MC} appear here the most homogeneous in their water quality diagnosis: 26 samples infer a weak pollution level and 8 samples a moderate one; however DI_{LM} seems to be

preferred because for the whole set of samples it displays a more narrow range of DI-values.

C O N C L U S I O N S

In the stream Lomme studied here the various sampled substrates were colonized by somewhat different diatom communities. This diversity in communities bias in some extent the calculated DI-values and consequently the diagnosis of water quality. Some diatom indices (DI_{MC} and chiefly DI_{LM}) display in this respect a more homogeneous estimation of the pollution level than others (DI_D and DI_S) and should be preferred.

Variability within natural substrates appeared also high and DI-value reckoned from a single sample may be misleading about the actual pollution level. Analysing more than one sample reduces the hazards of getting an extreme value; it is however time consuming and this feature may be considered as a shortcoming for a water quality monitoring method. Artificial substrate proved as a rule the most homogeneous.

Artificial substrate, - glass slides - , was colonized by diatom community which was not more different from communities on natural substrates than the latter were from each other; they thus may lead to fairly comparable index values. If in this study the slides give a little more optimistic estimation of stream water quality than the stones do, this feature is perhaps fortuitous. It is likely that during spring water quality was actually better than it could be inferred from natural substrate analysis, mainly from stones. ISERENTANT et BLANCHE (1986), in a transplantation experiment of colonized glass slides, pointed out the more rapid adjustment to new water conditions for communities growing on bare surfaces in comparison with already colonized substrates.

If on the one hand artificial substrates display a higher homogeneity in their diatom communities and if on the other hand they become colonized by communities seemingly better fitted to recent water conditions, we think they thus may be used as a good tool for routine measurements in monitoring programmes of stream water quality.

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Contamination of agricultural crops with toxic elements

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The plant production begins to be more and more influenced with the negative impacts of industrial activity. These impacts are direct and indirect. The direct operation of industry on the restriction of plant production volume consists in the soil reduction. The most distinct soil decrease shows to be in the area of surface coal mining and of developed energy activity. The mentioned activity results in large-volume wastes, i. e. spoil bank earths and power-plant fly-ashes which are being liquidated as a rule through the dumps. The large areas of these dumps (about 100 - 1,000 ha) require then biological recultivation in order to be included in a suitable manner into the regional ecosystem. Through the consequent agricultural recultivation of mine banks and fly-ash dumps we may compensate the decrease in agricultural soil and restrict in such a way the direct negative influence of industry on the plant production. The more effective method of recultivation will be chosen, the smaller losses will result for agriculture.

As the fundamental condition for a successful recultivation of the mentioned wastes with a very low level of biological activity we may regard their biological activating. For this purpose we have tested with an outstanding success the application of liquid manure - slurry (of swine, cattle and poultry), e. g. PETŘÍKOVÁ (1983). With slurry the nutrients necessary for plant vegetation have been simultaneously supplied. With application of very high doses of slurry on fly-ash dumps (up to 1,500 kg N . ha⁻¹) and also of medium-high doses of liquid manure on spoil banks - mine dumps (up to 300 kg N . ha⁻¹) we have secured appropriate vegetation conditions for immediate cultivation of cultural agricultural crops without any previous cultivation of the so-called pioneer plants. The evidence of that are the yields obtained from a direct recultivation of ash-fly dumps (without their covering with earths); the yields are presented in the Tab. 1.

Tab. 1. Yields of agricultural crops on the ash-fly dump to (cereal units per 1 ha).

I - lower dose, II - higher dose
Pi - slurry of pigs, C - of cattle
Po - of poultry

manuring variant	recultivation period			
	1 st year	a v e r a g e		
		1 st to 3 rd year	4 th to 7 th year	8 th to 10 th year
control	1.7	1.5	11.3	16.4
Pi I	17.0	24.6	30.8	44.6
Pi II	26.0	31.1	38.8	62.6
C I	18.8	27.9	33.6	45.4
C II	23.2	33.5	37.7	48.8
Po I	10.9	26.4	35.7	44.9
Po II	19.5	32.6	39.0	52.5

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The utilization of slurry to recultivation shows to have many advantages:

- biological activation of waste materials
- supply of vegetable nutrients
- effective utilization of excessive slurry and in such a way a restriction of its illicit liquidation which exerts otherwise an immediate negative influence on the environment
- acceleration of recultivation period on the spoil banks and ash-fly dumps
- possibility of immediate planting with verdure the waste dumps, also for the so-called temporary recultivation
- dustiness reduction in the environment of ash dumps.

In addition to the direct economic advantages, the utilization of slurry to the waste fertilization shows to have above all an indisputable ecological significance which consists in the rapid planting of non-productive wastes with cultural plants.

However, the observation of quality of the crops produced on these areas is of primary importance for the agricultural recultivation of waste substances. Therefore we concentrated our attention upon the examination of toxic elements, in particular of Cd, Hg, Pb and As. At present we have not at our disposal any explicitly reliable guidelines on the maximally allowable amounts of different in individual plant species (resp. organs as well). Therefore for an objective evaluation of analyses we took, in addition to the same plant species produced in the wastes, the same plants grown on the agricultural soil. The control samples (obtained from the soil) were two, on the one hand from the Chomutov district (the so-called "clean" area). This way of evaluation, on the basis of relative results, is fully in accordance with recommendations of GUDERIAN et al., 1970 that take it for the only correct and objective one. The evaluation of existing results (for five years) has shown that the highest content of toxic elements has not been always revealed in plants grown on wastes, even when it was a case of their so-called direct recultivation, without any covering of the dump surface with the earth. This follows from the summary Tab. 2 presenting average values which were obtained from the analyses of cereals (grain and straw), maize, clover-grass, luzerne, potatoes, sugar-beet, green sunflower and some legumes.

Tab. 2. Content of toxic elements in agricultural crops in ppm (average of five years).

element	locality			
	fly-ash	spoil bank	agricultural soil	
			district Chomutov	district Benešov
Cd	0.09	0.12	0.19	0.15
Hg	0.010	0.064	0.022	0.013
Pb	6.3	5.1	4.2	5.7
As	6.0	3.5	7.2	3.5

Analyses of different cereal species (grain and straw), potatoes, sugar-beet and green maize, which created integrated sets, were evaluated statistically with the variance analysis. Between different localities no statistically significant difference was recorded (except for the Pb content in the rye straw where, however, the content was low, maxim. 1.0 ppm Pb).

From this fact we can draw conclusions that the plants grown on wastes (including fly-ash) within the scope of our experiments are not of inferior quality as compared with plants produced on agricultural soil.

The industrial activity exerts an indirect influence on the plant production as told in introduction. Above all immissions belong to indirect influences which can work quantitatively (decrease of yields) as well as qualitatively (contamination of crops); in addition to the direct injuries to agricultural crops, the immissions can damage even the soil which may be then a further potential source of danger for crops grown on such a soil. The source of plant contamination is thus on the one hand the polluted atmosphere and on the other the soil. The portion of these two fundamental sources of plant contamination with foreign matters has not yet been reliably determined. The motion of the most of heavy metals in the soil is being limited because they create complicated substances with soil colloids and organic matter as it is generally known from the literature (e. g. KABATA - PENDIAS, 1984). These properties can be found above all in Pb and Hg of four most substantial toxic elements. It looks different in case of Cd (and also As) which seem to be more mobile in the soil and are therefore easier accepted through the plants (from substrate in which they are cultivated). For an important source of plant contamination we can thus take the polluted atmosphere. Therefore we have concentrated our attention on the determination of a content of some heavy metals in the meadow forage harvested from permanent grass stands in submontane region of Ore Mountains. It is a case of the region where the forest cultures are considerably injured and where consequently the negative influence of immissions has been explicitly demonstrated. The plant samples were analysed in 1985, in the year of extreme drought, mainly in the course of vegetation. In relation to precipitations we calculated the correlation coefficient and represented regression lines of the Cd, Pb and As contents in the hay samples from this submontane region as the following Fig. 1.

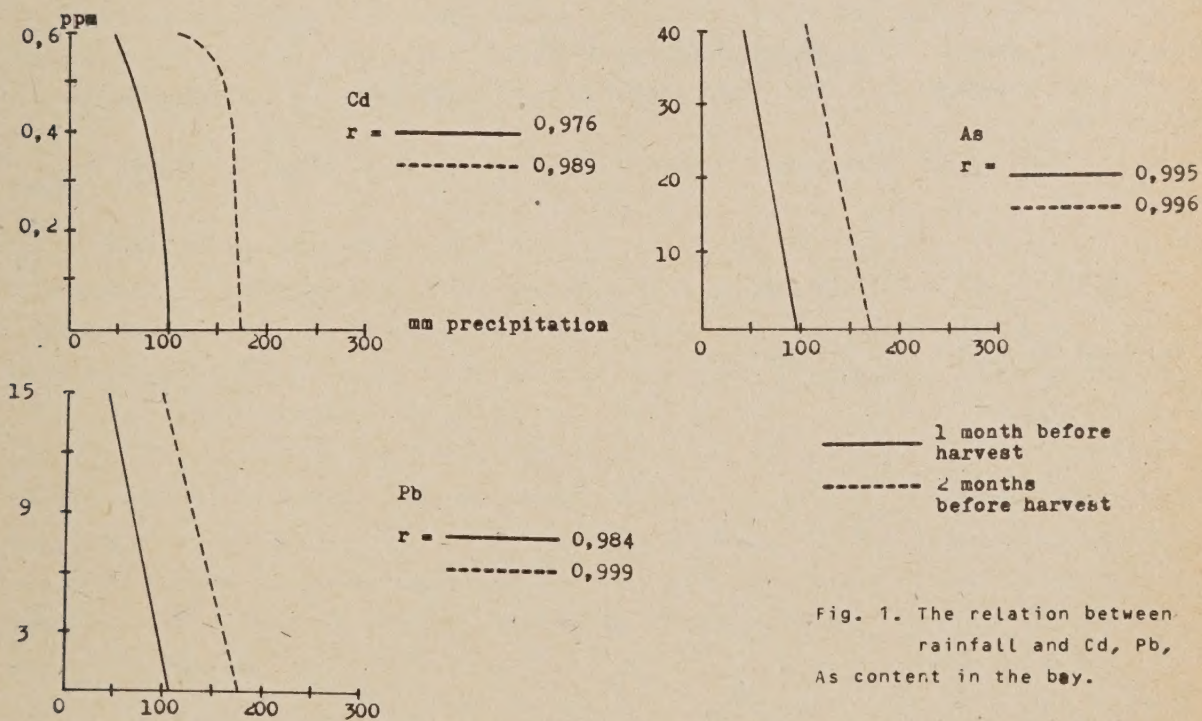


Fig. 1. The relation between rainfall and Cd, Pb, As content in the hay.

The direction of regression lines has quite clearly indicated that the degree of plant contamination with all three elements under study is dependent on rainfall the lower precipitations, the higher content of these elements has been found in plants. It means that solid immissions, which are retaining on the plant surface and are not washed away by the rain, become the principal contamination source of plants with toxic elements. The evaluation of these results shows that the polluted atmosphere will be an important contamination source of plants with undesirable elements. Contamination intensity of agricultural plants depends on plant species too. We found out, that content of Cd, Pb, Hg and As in plants was the minimal in cereals, the maximal in fodder crops. It is evident from this view:

Succession	1	2	3	4	5	6	7	8
Crop	grain		straw	potato	sugar beet	lucerne	maize	grass + clover
	peas	cereals						

Among cereals the gighest content of toxic elements was found in barley, the lowest in wheat.

The results obtained from the analyses of plants grown on wastes (see above) give evidence on the fact that in the plant contamination the direct pollution of external plant surface (from the air) shows to have a higher portion than the uptake of foreign matters by the root system of plants from the soil (or other substrate - including wastes). Therefore it will be particularly important to pay attention to fundamental restriction of emmissions from industrial enterprises and plants into the atmosphere. The problems of immission influence on the plant production have not yeat been sufficiently examined and there are not satisfactory concrete results necessary to compensation of harmful influences induced by industry.

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Der Wurzelwuchs als biologischer Anzeiger für die toxische Wirksamkeit der Schwermetalle im Boden

A. BALUK

E I N L E I T U N G

Die Folge der Emission um Hüttenwerke kann die Akkumulation der Schwermetalle im Boden sein. Die für die Pflanzen unschädlichen Schwermetallgehalte werden in Grenzzahlen angegeben (KLOKE, 1974). Diese Zahlen widerspiegeln jedoch schwach die aktuelle toxische Wirkung dieser Metalle für die Pflanzen, weil sie die Phytotoxizität determinierenden Faktoren nicht in ausreichenden Grad berücksichtigen. Die wesentliche Rolle spielt hier pH des Bodens, Löslichkeit einzelnen Schwermetalls in den Bodenwasserlösungen, granulometrische Bodenzusammensetzung und Gehalt der organischen Substanz (HODENBERG, 1974; KABATA-PENDIAS, 1976, 1979; HERMS, BRUMMER, 1980; BRUMMER, HERMS, 1983; BALUK, 1985). Bei der Bestimmung der Schadzonen können die Anzeigerpflanzen behilflich sein (STEUBING, 1981). Als Bioanzeiger der Wirkung von Schwermetallen waren in meiner Methode keimende Samen.

M A T E R I A L U N D M E T H O D I K

Die Keimung wurde im Kristallisator (ø 9 cm, Höhe 5 cm) mit Glas bedeckt, im Temperatur 20 - 22° C durchgeführt. Der Untergrund bildete Filterpapier mit 10 cm³ wässriger Lösung des Schwermetallsalzes befeuchtet, oder 40 g zur völligen Wasserkapazität gebrachten anlehmigen Sandes. Der erwünschte pH-Wert wurde durch Zugabe von H₂SO₄ oder Ca(OH)₂ und der Schwermetallgehalt durch entsprechende Menge seines Salzes erreicht. Die Samen (20 Stück) wurden nach 72 Stunden Umrühren und Inkubation des Bodens mit Metall gesät. Nach 6 Tagen der Keimung wurde die Länge von der Keimwurzel und vom Stengel gemessen und die Gesamtmasse der Wurzeln und des Stengels gewogen. Zusätzlich wurden auch die Gefäßuntersuchungen gemacht, in denen auch von Ackerkrume genommener anlehmiger Sand verwendet wurde.

E R G E B N I S S E

Die Rapssamen keimten auf wässriger Lösung und auf Untergrund mit differenzierten Schwermetallgehalt in ähnlichem Prozent wie auch ohne deren Zugabe. Die Unterschiede traten v. a. im Keimwurzelwuchs auf. Die Hemmung des Stengelwuchses war mit grösserer Konzentration des Kupfers in Lösung weniger deutlich und auch kleinere Unterschiede gab es in von Wurzeln und Stengel erzeugter Frischmasse (Tab. 1).

Die Differenzierung der pH-Werte von wässrigen Lösungen von 4.0 bis 7.0 bewirkte keine wesentlichen Unterschiede im Wuchs der Rapswurzel und -stengel. Die Differenzierung der pH-Werte des Bodens bei der Keimung 12 verschiedener Samenarten bewirkte jedoch verschiedenen Effekt der Wirkung steigenden Schwermetallmengen, v. a. Kupfer und Zink. Die hemmende Wirkung von Kupfer und Zink auf den Wuchs der Keimwurzeln bei pH-Wert 5.0 war wesentlich stärker als bei pH-Wert 6.5 (Tab. 2).

Tab. 1. Einfluss steigender Cu - Konzentrationen in der Wasserlösung auf die Wurzellänge, Stengelhöhe und den Raps-Keimlingseertrag (nach 6 Tagen der Keimung)

Cu mg/dm ³	Wurzellänge		Stengelhöhe		Frische Masse Wurzeln + Stengel	
	mm	%	mm	%	mm	%
0	83,4	100	38,0	100	1,42	100
2	39,4	47,2	35,0	92,1	1,20	84,5
5	21,1	25,2	30,8	81,1	1,20	84,5
10	11,5	13,8	30,0	78,9	1,11	77,5

Auf dem zum Versuch verwendeten anlehmigen Sand hemmte die Kupfermenge 50 mg/kg, besonders bei pH-Wert 6,5, bei meisten untersuchten Pflanzen-Arten noch schwach den Wurzelwuchs. Dagegen die Kupfermenge 100 mg/kg verursachte sehr deutliche Verkürzung der Keimwurzel. Diese Verkürzung betrug bei Raps bei pH Wert 5,0 sogar 87 %. Diese Menge wirkte bei pH-Wert 6,5 wesentlich schwächer. Bei Roggen, Mais, Salat, Flachs, Klee und Wicke fehlte deutliche, den Wurzelwuchs hemmende Wirkung oder deren Verkürzung überschritt 35 % im Vergleich zur Kontrolle nicht.

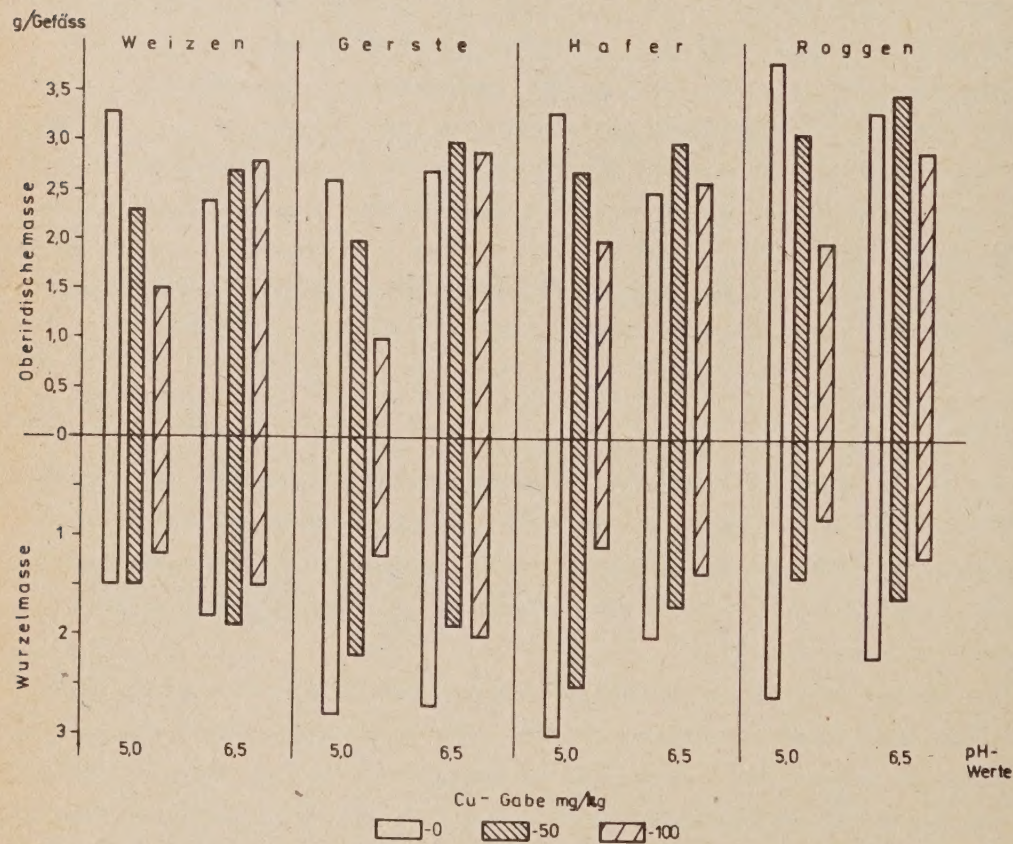


Abb. 1. Trockene Getreidemasse am Bestockungsende.

Tab. 2. Einfluss der pH-Werte des lehmigen Sandes und der Schwermetallkonzentration auf die Wurzellänge verschiedenen Pflanzenarten, 0 Abweichungen zur Kontrolle $\pm$ 15 %, - Verminderung (+ Vergrößerung) der Wurzellänge 16-35 %, -- 36-55 %, --- > 55%.

Pflanzen- arten	pH- Werte	Metallkonzentration in mg/kg des Bodens							
		Cu		Zn		Cd		Pb	
		50	100	100	300	18	54	800	2400
Roggen	5,0	-	--	0	--	0	0	0	-
	6,5	0	-	-	-	0	-	0	0
Weizen	5,0	--	---	0	---	0	0	0	-
	6,5	0	--	0	0	0	-	0	-
Gerste	5,0	-	---	0	---	0	-	+	-
	6,5	--	---	0	-	0	--	0	-
Hafer	5,0	--	---	0	--	-	--	-	--
	6,5	0	--	0	-	0	-	--	--
Mais	5,0	0	--	-	0	-	0	0	-
	6,5	0	0	0	0	0	0	0	-
Italienisches Raigras	5,0	0	---	0	--	0	0	-	---
	6,5	0	0	-	-	0	0	0	-
Salat	5,0	0	--	0	---	-	-	-	---
	6,5	0	++	+	+	0	0	0	-
Klee	5,0	0	0	-	---	0	0	-	--
	6,5	0	-	--	0	0	0	-	-
Lein	5,0	0	0	+++	-	+++	++	+	-
	6,5	++	0	++	+	0	++	0	-
Wicke	5,0	0	--	0	---	-	--	--	---
	6,5	-	0	-	0	-	-	0	-
Raps	5,0	-	---	0	---	-	-	-	-
	6,5	0	-	0	0	0	0	-	0
Zucker- rübe	5,0	0	0	--	---	--	---	-	---
	6,5	+	0	-	--	+	0	0	--

Das Zink verursachte ähnliche Hemmung vom Keimwurzelwuchs wie Kupfer aber bei paarmal höheren Mengen. Bei pH-Wert 6,5 wurde keine negative Wirkung auf Wurzelwuchs bei Weizen, Mais, Salat, Klee, Flachs und Raps festgestellt. Bei Flachs wurde sogar positive Zinkwirkung auf Keimwurzelwuchs registriert. Bei pH-Wert 5,0 wurde starke, den Keimwurzelwuchs hemmende Wirkung bei 300 mg/kg Zink bei Weizen, Gerste, Salat, Klee, Wicke, Raps und Zuckerrüben festgestellt.

Das Kadmium in Menge 18 mg/kg bewirkte geringe Hemmung des Wuchses nur bei einiger Pflanzen. Am empfindlichsten gegen erhöhte Kadmium-Gehalte im Boden zeigte sich Zuckerrübe. Die erhöhten Kadmium-Gehalte im Boden wirkten positiv auf Keimwurzelwuchs bei Flachs.

Das Blei in Menge 800 mg/kg verursachte generell keine deutlich negative Wirkung auf Keimwurzelwuchs untersuchter Pflanzen. Erst bei der Menge 2400 mg/kg bei pH-Wert 5,0 erfolgte deutliche Hemmung des Keimwurzelwuchses bei Italienischem Raygras, Salat, Wicke und Zuckerrübe.

Tab. 3. Einfluss steigender Zn-Gaben auf Ertrag Italienisches Raigras (Gefässversuch, anlehmiger Sand, pH-Werte 5,0).

Zn-Gaben (mg/kg)	Oberirdische trockene Masse (g/Gefäss)			Gesamtertrag		Trockene Wurzelmasse
	I	II	III	g	%	%
0	9,0	4,8	0,9	14,7	100	100
75	9,3	4,4	0,9	14,6	99	79
300	9,2	2,0	0,5	11,7	80	37

Die hemmende Wirkung von erhöhten Zinkgehalte im Boden auf Pflanzenwuchs wurde auch in Gefässuntersuchungen mit Italienischem Raigras festgestellt (Tab. 3).

Die Differenzierung der oberirdischen Masse nahm infolge sich erhöhenden Zinkmengen im Ertrag des zweiten und dritten Schnittes zu. Die Wurzelmassereduktion war ca. dreimal grösser als der oberirdischen Masse.

Die Ergebnisse mit Kupfer wurde in Abb. 1 festgestellt. Die sich erhöhenden Kupfermenge im Boden verursachten am Ende der Bestockung deutliche Unterschiede in erzeugter Masse nur bei pH - 5,0. Die Wurzeln wuchsen dabei flächer, aber sie waren stark verzweigt (Abb. 2). Bei pH-Wert 6,5 waren die Unterschiede besonders in Wurzelmasse nur bei Roggen und Hafer deutlicher. Die Ergebnisse der Untersuchungen zusammenfassend, kann man feststellen, dass die Differenzierung im Pflanzenwuchs infolge erhöhter Kupfer- und Zinkgehalte im Boden während der Keimung findet auch in weiteren Entwicklungsphasen der Pflanze statt. Die erlangten Ergebnisse nach 6 Tagen der Keimung können als Anzeiger des Grades negativer Wirkung erhöhter Kupfer- und Zinkgehalte in untersuchten Bodenproben dienen. Der empfindlichste Anzeiger zu diesem Zweck ist Raps. Für erhöhte Kadmium-Gehalte im Boden Kann Zuckerrübe Bioanzeiger und für Blei - Salat, Italienisches Raigras, Wicke und Zuckerrübe sein.

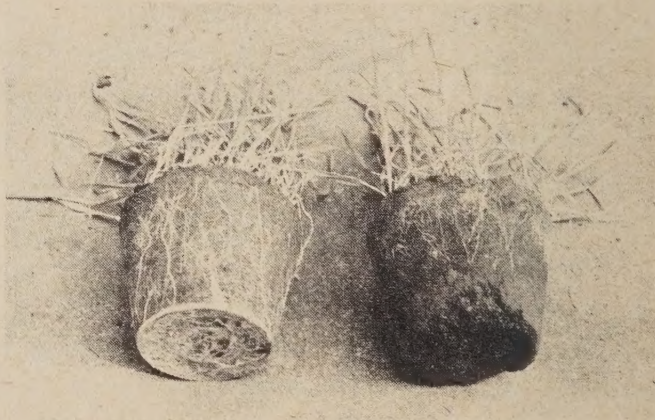
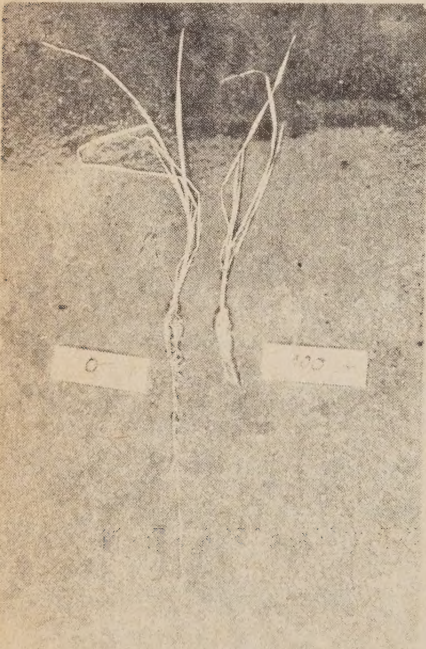


Abb. 2. Das Wurzelwerk von Roggen bei pH-Wert 5.0.

Links - ohne Cu - Gabe
rechts - 100 mg Cu/kg

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Contents of some metals in Salicaceae leaves of technogenical ecotopes

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Species of Salicaceae are characterized by a high rate of growth, resistance to pollutants, pollutant-accumulation, extremely high expansion ability, considerable ecologic and evolutionary differentiation (SKVORTSOV, 1968; SIDOROVICH et al., 1981; KULAGIN, 1982). Contents of Ti, Mn, Fe, Co, Cu, Rb, Sr in leaves of *Salix* and *Populus*, growing under urban conditions, by the main roads, on copper-quiver and iron-ore deposit dumps of Pre-Ural and South Ural areas were determined. The species are arranged according to the degree of metal accumulated (metal content coefficients):

Ti	P. alba 1	P. tremula 1.02	P. nigra 1.12	S. triandra 1.14	S. alba 1.4
Mn	S. triandra 1	S. alba 1.2	P. alba 2.3	P. tremula 4.3	P. nigra 6.8
Fe	P. alba 1	S. triandra 1.5	P. nigra 1.7	P. tremula 2.6	S. alba 2.7
Co	P. alba 1	P. nigra 1.3	S. triandra 1.4	P. tremula 1.9	S. alba 2.0
Cu	S. alba 1	P. tremula 1.04	S. triandra 1.05	P. alba 1.086	P. nigra 1.087
Rb	S. triandra 1	P. tremula 1.2	P. alba 1.3	S. alba 1.5	P. nigra 2.2
Sr	S. triandra 1	P. alba 1.1	P. nigra 1.2	P. tremula 1.6	S. alba 2.9

The contents of Cu and Ti were relatively similar in the species studied, while a broad variation in Mn, Sr and Fe levels in *Salix* and *Populus* were observed. The highest metal accumulation was revealed in *P. nigra* (Mn - 4.8 mg/kg, Rb - 32), *S. alba* (Fe - 878; Co - 0.43). Species of Salicaceae can be used as indicators of pollution and as phyto-filters of metals.

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Effect of cadmium on the growth of bean (*Vicia faba*)

K. MONDSPIEGEL

INTRODUCTION

The problem of accumulation of trace elements in biological matter is attracting ever-interest. Among the most hazardous toxic elements are heavy metals, of which cadmium has been proved to have carcinogenic and mutagenic effects. Natural concentrations of cadmium are estimated to $2 \mu\text{g}/\text{m}^3$, its concentrations in soil can be as high as 4.5 ppm. This rather high cadmium concentration in soil may be due to its presence in matter undergoing humification (BROOKS, 1977).

In industrial areas, in the vicinity of local sources of pollution or traffic arteries, the concentrations of this element in air and soil are higher. Plants can take up cadmium by their leaves from the air and, to a considerably higher extent, by their roots from the soil. From plants, cadmium passes further to the next trophic chain links. Given the high immission fall-out, toxic substances accumulate in soil where they can directly affect plant production and deteriorate the quality of plants of importance to man (ANONYMUS, 1970).

Cadmium concentrations in soil are highest in the 0 - 5 cm horizon; deeper, the cadmium content of soil decreases with increasing depth. The degree of soil pollution by cadmium is affected by the presence of zinc. In little polluted regions the zinc-to-cadmium ratio is estimated to 300 - 700, in highly polluted urban areas this ratio is 100 - 300 in soil in 0 - 5 cm depths (CZARNOWSKA, 1980).

Increased cadmium levels in soil stimulate its increased content of plants (CZARNOWSKA, 1974). Bioavailability of cadmium from soil depends on many factors, the most important of which are the plant species, soil pH, redox potential and composition of the soil solution (BINGHAM et al., 1986).

During the growing of pin oak (*Quercus palustris*) in a sand culture with a complete nutrient solution free from cadmium or containing CdCl_2 in a concentration of $0.1 \mu\text{g}/\text{ml}$, accumulation of cadmium was proved particularly in the roots. During a chronical test, cadmium also entered the above-ground parts of the plants, its amount in the leaves, however, was as much as 11 times lower than in the root system (KAZIMIR, BRENNAN, 1986). In a chronical test with CdCl_2 on barley, the cadmium accumulation ratio in the above-ground-to-root parts was 1 : 14 (LANGERWERFF, 1971). The effect of cadmium and lead on the growth and biomass of forest vegetation was studied in natural conditions (BACKHAUS, BACKHAUS, 1987). A significant negative correlation between the lead content and biomass of roots was found, whereas for cadmium a positive correlation was only proved in a certain age category of the forest stand.

Attention has also been paid to the distribution of heavy metals in plants. KABATA-PENDIAS (1977) and STEPANOVA (1980) observed that stems and leaves accumulated heavy metals in lower amounts than roots did, which suggests that roots act as a protective barrier preventing toxic metals from passing on to the above-ground parts of plants. Similar results were also obtained by KAZIMIR et BRENNAN (1986) and by LAGERWERFF (1971) for cadmium in chronical tests. The data by MEMON (1980) suggest that cadmium contents of 0.05 - 0.6 mg/kg dry weight are harmless to the plant network.

In the present work, accumulation of cadmium was examined in a laboratory experiment using bean (*Vicia faba*) as the test plant, with the aim to seek whether this metal is uniformly distributed in the plant or it is taken up preferentially by some particular plant organs; in particular, whether the roots have a tendency to retain a higher fraction of this element during its transport to the above-ground parts of the plant. The effect of cadmium on the growth of bean and the amount of the produced biomass was also investigated.

M A T E R I A L A N D M E T H O D

Seeds of bean were allowed to germinate in Petri dishes using Knopp's nutrient solution (ČVANČARA, 1962) which contained cadmium sulphate in concentrations of 0, 0.25, 2.5, 25 and 250 ppm. The pH of the hydroponic solutions was 4.6 - 4.8. Each dish accommodated 10 bean seeds on filter paper. The level of the solution was held constant by repeated replenishing with distilled water. The 5th day the plants were transferred to a hydroponic solution of the same composition as used for the germination.

The hydroponic device comprised a glass vessel 460 ml volume and a plastic pot adapted to accommodate 10 plants. The glass was kept in dark by using a black foil. The nutrient solution level was again held constant by additions of distilled water. The light regime was L : D = 16 : 8, temperature $22 \pm 1^{\circ} \text{C}$, humidity $60 \pm 5 \%$.

The 15th day the plants were taken out and their roots were cleaned in distilled water. Each set of plants (10 specimens) from a given concentration was handled and evaluated separately. The root systems, stems and leaves were separated and their biomass was evaluated after drying to constant weight. The cadmium content was determined by atomic absorption spectrophotometry using an Instrumentation Laboratory Model 457 spectrophotometer.

Statistical significance of the data was evaluated by means of Duncan's test. The number of sets for biomass evaluation (10 plants each) was 6, 7, 9, 8 and 9 for cadmium concentrations in the nutrient solution 250, 25, 2.5, 0.25 and 0 ppm, respectively. The biomass values for the individual cadmium concentrations were compared with the control data; their mutual differences were evaluated as well. The growth of the plants (their height) was evaluated likewise for the different cadmium concentrations.

R E S U L T S

Plant Growth and Biomass Produced

Tab. 1 demonstrates that the biomass of the root system and leaves decreases significantly with increasing concentration of cadmium in the hydroponic solution. For stems, the differences in the biomass from cadmium concentrations of 0, 0.25 and 2.5 ppm are statistically insignificant, and only for cadmium concentrations of 25 and 250 ppm the biomass is significantly different. The plant height decreases with increasing cadmium concentration.

Application of Duncan's test gave evidence that the effect of cadmium on the biomass of the roots, leaves and stems and on the plant growth is statistically significant.

At the $\alpha = 0.05$ confidence level the root biomass from each of the cadmium concentrations (250, 25, 2.5 and 0.25 ppm) is significantly different from the other sets and from the control,

only for the concentrations of 2.5 and 0.25 ppm the mutual biomass difference is statistically insignificant.

The biomass of the leaves was evaluated at the $\alpha = 0.01$ level. The values for cadmium concentrations of 250 and 25 ppm do not differ significantly from each other but differ from the control and from the values for cadmium concentrations of 2.5 and 0.25 ppm. The two latter also differ significantly from the control, and also from each other.

At the $\alpha = 0.01$ level the stem biomass from cadmium concentrations of 250 and 25 ppm differs significantly from the control and the remaining sets but not from each other. From the cadmium concentrations of 2.5 and 0.25 ppm the stem biomass differs significantly neither from the control nor from each other.

The plant growth was also evaluated at the $\alpha = 0.01$ confidence level. The plants from the cadmium concentrations of 25 and 2.5 ppm differ significantly from the control and the remaining sets as well as from each other. The height of plants from cadmium concentrations of 2.5 and 0.25 ppm differs significantly from the control but not from each other.

Cadmium Accumulation in Plant

The cadmium concentrations in the individual parts of the plant are given in Tab. 2. The total amount of cadmium in the plant increases with its increasing concentration in the hydroponic solution. The amounts are highest in the root system whereas in the leaves and stems the cadmium concentrations are several times lower. The correlation coefficient values document a close correlation of the individual plant organs and its concentration in the nutrient solution ($r = 1.00, 0.99$ and 0.99 for roots, stems and leaves, respectively).

D I S C U S S I O N

The results of the tests are consistent with the assumption that the cadmium toxicity threshold is about 1 mg/kg dry plant weight. In view of the biomass decrease and cadmium content of leaves at concentrations in the hydroponic solution as low as 0.25 ppm Cd, it can be assumed that some physiological disorder occurs in the bean at this concentration. Concentrations in excess of 1 ppm are observed to affect negatively both the biomass and plant growth. At cadmium concentrations in the hydroponic solution of 25 and, in particular, 250 ppm, pathological changes occur in the plant, whereupon the plants frequently cease to grow and die. A limit at which the plants do not die but their growth is slowed down is 10 ppm Cd in the nutrient solution.

The results agree with the conclusion of other authors that plant yields can be lower in industrial regions. Although many factors affecting the bioavailability of cadmium and other heavy metals operate in nature, the final outcome is a negative effect on the agricultural produce anyway, similarly as was proved in this experiment for bean.

S U M M A R Y

In a laboratory experiment concerned with the effect of cadmium on bean (*Vicia faba*), evidence was obtained that this element can accumulate in plant bodies. Cadmium absorbed is not

Tab. 1. Effect of cadmium on the biomass of the individual parts of bean and on the plant growth.

Cd concentration in nutrient solution ppm	Biomass, g dry weight						Plant length cm	
	roots		leaves		stems		$\bar{x}$	s_x
	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x		
0	0.522	0.044	1.069	0.090	0.837	0.131	22.17	4.54
0.25	0.438	0.053	0.899	0.128	0.695	0.181	18.81	5.57
2.5	0.396	0.076	0.753	0.057	0.795	0.179	17.58	4.84
25	0.128	0.035	0.118	0.041	0.176	0.069	3.00	2.93
250	0.058	0.016	0.039	0.006	0.082	0.016	0.58	0.81

Tab. 2. Cadmium contents of individual bean parts.

Cd concentration in nutrient solution ppm	Cadmium content, ppm					
	roots		stems		leaves	
	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
0	0.89	0.21	0.34	0.26	0.05	0.03
0.25	134.95	56.90	10.23	2.95	6.44	0.65
2.5	624.69	102.21	95.70	20.78	47.26	8.95
25	6 369.79	1 368.42	395.90	132.90	89.76	14.39
250	43 529.90	3 271.05	5 039.35	220.76	2 387.63	320.71

distributed uniformly in the plant: the concentration in the root system is several times higher than in the above-ground parts of the plant. This bears out the suggestion that plant roots can act as a protective barrier against this metal. The biomass of both the roots and above-ground parts of the plant is the lower the higher is the concentration of cadmium in the nutrient solution. Positive effect of cadmium on the bean growth was never observed.

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Ecophysiological changes caused by heavy metals in wheat seedlings

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Of the pollutants of our environment, heavy metals have become increasingly hazardous and may raise ecophysiological problems in plants.

Differential toxicity symptoms of different plants were demonstrated by LEE et al. (1976), CARLSON et al. (1976), KOEPPE (1977), LAMOREAUX, CHANEY (1978), MAHLER et al. (1978), ERNST (1980), TOBART et al. (1985). Plants have shown to have accumulated heavy metals in vegetative tissues and seeds (PELOSI et al., 1976; CATALDO et al., 1978; WALLACE, 1977a; McBRIDE, 1981; VALDARES et al., 1983).

Many of the heavy metals are known to affect the amount and the relative proportion of micro elements in plants (CSEH, BUJTÁS, 1980; WALLACE et al., 1976; WALLACE, 1977b, c, d; FOY et al., 1978; VELTRUP, 1981; GIRLING, PETERSON, 1981; CLARKE et al., 1982).

These results called our attention to the importance of micro elements respective to the absorption, and certain interactions, and the possibilities of bioindication. Therefore the following experiments were done to investigate the reduction of assimilating surface, the heavy metal and the essential micro element content and proportion of wheat seedlings. The effect of nitrogen nutrition has also been examined on the heavy metal toxicity.

MATERIAL AND METHODS

Wheat seedlings (*Triticum aestivum* L. cv. MV 8) were used for experiments. Seeds were soaked for ca. 12 h at room temperature, and germinated in darkness for 36 h, at 16°C. After this two days, the seedlings were exposed to the effect of heavy metals that is, were placed on 500 ml plastic culture pots. The plants were maintained in controlled environment chambers (typ. E 15) with a 16/8 hr light cycle with 7500 Lx, a day/night temperature cycle of 16/14°C, and 85 % RH.

The nutrient solution contained $1.35 \cdot 10^{-3}$ M KCl, $0.45 \cdot 10^{-5}$ M CaCl₂, $0.5 \cdot 10^{-3}$ M MgSO₄ · 7 H₂O, $0.22 \cdot 10^{-3}$ M KH₂PO₄, $0.77 \cdot 10^{-3}$ Ca(H₂PO₄)₂, and Hoagland AZ nutrient solution (50 ml/1000 ml). We used NH₄NO₃ for nitrogen supply of 10^{-5} , $5 \cdot 10^{-5}$, 10^{-4} , $5 \cdot 10^{-4}$, 10^{-3} , and $5 \cdot 10^{-3}$ M concentrations. Four heavy metals (Cd, Cr, Ni and Pb) in nitrate salt form were used during the experiments. (The nitrogen content of metal salts were taken into consideration.) The pH was adjusted to 5 - 5.2. The solution were not changed during the growth period. There were 6 replications.

Fourteen days after seeding, the shoots were measured by an automatic area meter (Model AAM-7) and 26 elements of the shoot material were measured by an ICAP - 9000 typ. argon plasm spectrometer.

RESULTS AND DISCUSSION

Effect of heavy metals on the assimilating surface

Measurements of the wheat seedlings showed that Cr^{3+} ions inhibited the growth of shoots. The reduction of the assimilating surface was about 60 - 65 % up to the $5 \cdot 10^{-5}$ M Cr treatment level. By the nitrogen nutrition the adverse effect of Cr could be decreased at this Cr concentration. Further increasing the Cr level there were not any positive effects of nitrogen nutrition. The growth reduction was more than 85 - 90 % (Fig. 1).

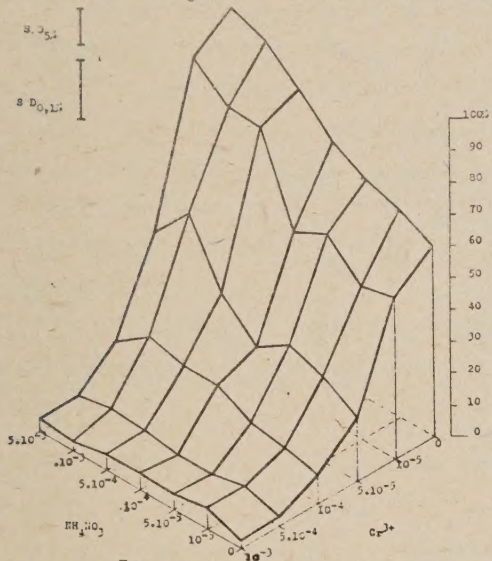


Fig. 1. Cr^{3+} and NH_4NO_3 effect on the assimilating surface of wheat seedlings.

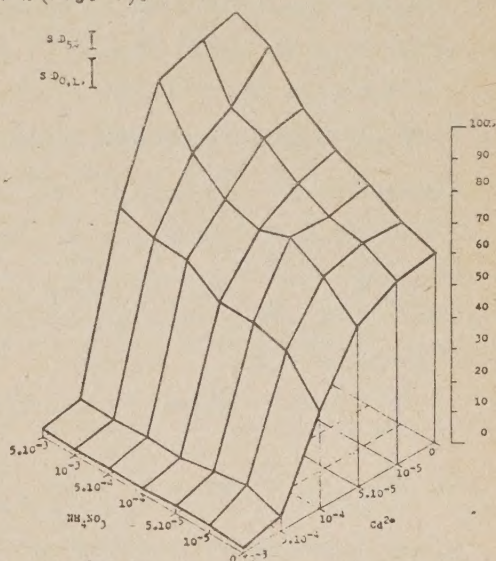


Fig. 2. Cd^{2+} and NH_4NO_3 effect on the assimilating surface of wheat seedlings.

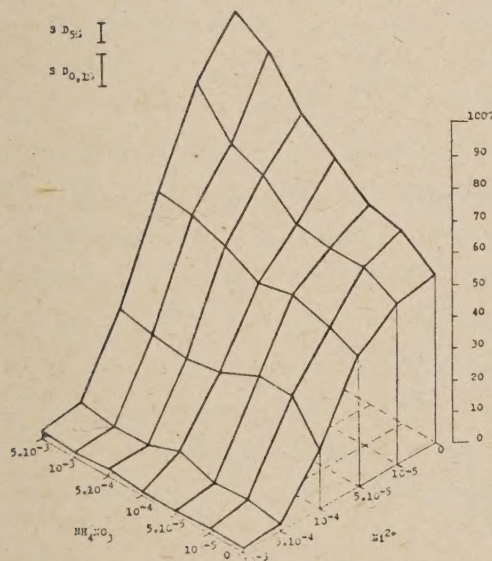


Fig. 3. Ni^{2+} and NH_4NO_3 effect on the assimilating surface of wheat seedlings.

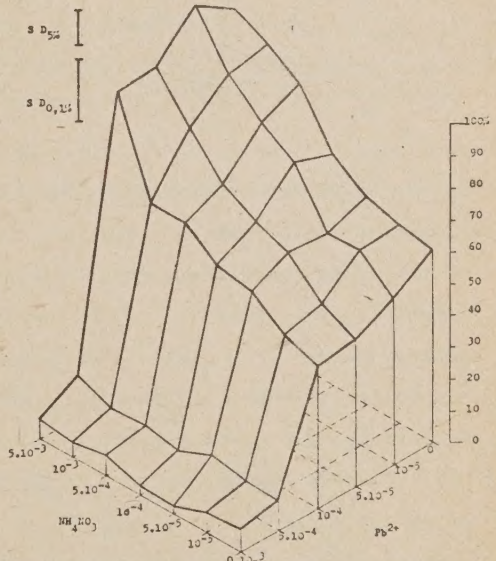


Fig. 4. Pb^{2+} and NH_4NO_3 effect on the assimilating surface of wheat seedlings.

The adverse effect of Cd treatment up to the $5 \cdot 10^{-5}$ M concentration was relatively low not more than 5 - 15 % (Fig. 2):

Ni toxicity followed nearly the same pattern with Cd. By increasing the NH_4NO_3 level we realised a 25 - 27 % growth up to $5 \cdot 10^{-5}$ M Cd and Ni concentration. Beyond the 10^{-4} M metal concentration we could not achieve growth with nitrogen increase (Fig. 3).

The effect of Pb up to the 10^{-4} M treatment level was low, not more than 7 - 15 % decrease of the assimilating surface. Pb showed a strong reduction in the growth beyond the $5 \cdot 10^{-4}$ M Pb concentration. Measurements showed a reduction of 88 - 90 % of the assimilating surface, with no buffering effect (Fig. 4).

Effect of heavy metals on the element content

Each treatment level of Cd increased the Cd content of shoots. Up to the 10^{-4} M of Cd content of nutrient solution, treatments did not significantly differ from each other. The Ce content of the plant significantly increased at concentrations of $5 \cdot 10^{-4}$ and 10^{-3} M Cr. Ni treatments increased the Ni content of wheat seedlings. Significant difference could be seen at $5 \cdot 10^{-4}$ M and 10^{-3} Ni concentration of the nutrient solution. Pb treatment increased the Pb content of shoots, but in a smaller amount than the other heavy metals (Tab. 1).

Tab. 1. Cd, Cr Ni and Pb content of wheat shoots.

Concentration of metal in the nutrient solution (M).	<u>µg/g dry weight</u>			
	Cd ²⁺	Cr ³⁺	Ni ²⁺	Pb ²⁺
0	11.90	4.92	2.71	2.57
10^{-5}	28.68	6.25	18.89	16.75
$5 \cdot 10^{-5}$	118.13	24.24	65.93	36.98
10^{-4}	239.79	41.82	112.03	35.87
$5 \cdot 10^{-4}$	671.81	255.45	372.75	17.10
10^{-3}	1878.20	926.13	1864.33	65.26

In the course of our experiment we have measured 26 elements in the shoots. Here we can give only some details from our results regarding certain data of essential micro elements. According to the international literature and practice, we restricted the range of essential micro elements to six elements, B, Zn, Mn, Mo, Cu and Fe.

Cd tratment up to the $5 \cdot 10^{-5}$ M Cd concentration increased the B content, but the $5 \cdot 10^{-4}$ and 10^{-3} M Cd level in the nutrient solution decreased the B content significantly. Cr tre- atment decreased the B content by 40 - 60 %. Ni concentration decreased the B contnet of shoots. Pb treatment had no significant effect on B content up to $5 \cdot 10^{-4}$ M Pb concentration. 10^{-3} M of Pb increased the B content.

Cd treatment increased the Zn content, but only over $5 \cdot 10^{-4}$ M Cd level was significantly different. Cr treatment increased the Zn content. In the case of Ni treatment, $5 \cdot 10^{-4}$ M Ni concentration in the nutrient solution increased the Zn content. Pb had a similar, but mild effect to that of Ni.

At the 10^{-5} , $5 \cdot 10^{-5}$, and 10^{-4} M Cd concentration the Mo content increased, at $5 \cdot 10^{-4}$ and 10^{-3} M Cd level the Mo content decreased with 70 - 80 %. 10^{-5} M Cr concentration had a little effect on the Mo content of shoots. The different levels of Ni concentration reduced the Mo content. Pb treatment reduced the Mo content as well.

At 10^{-3} M Cd concentration Cu content increased. Cr treatment had no effect on the Cu content. At the high Ni concentrations the Cu content increased. Pb like Cr content had no significant effect on the Cu content.

Cd concentration increased the Fe content, but had no significant effect, except at 10^{-3} M Cd concentration. Cr treatment reduced the Fe content at lower concentrations, but at $5 \cdot 10^{-4}$ and 10^{-3} M Cr level the Fe content increased. Up to the $5 \cdot 10^{-4}$ M Ni concentration there was no significant effect on the Fe content. 10^{-3} M Ni concentration increased the Fe content. Pb treatment had no significant effect, except at high concentration (Tab. 2).

Treatment levels (included the control as well) were compared to one another with regard to all measured elements, by use of cluster analysis. Objects, to be classified were the treatment levels, while the attributes on which the comparison was made were the element concentrations measured in shoots. We counted up the Czekanowski's comparative function, and we used the "farthest neighbour" method.

The dendrogram (Fig. 5) shows dissimilarity of the treatments in the case of each metal. Legs of dendrograms mean the heavy metal treatments.

The horizontal lines between the legs or clusters show the degree of similarity or distance.

As an example in case of Cr treatment we can see that the control is the least similar to the other treatment, that is due to any kind of Cr concentration, the content of the majority of the elements changed in the plants, since analysis took the full element content into consideration. (If only one or two element concentrations changed, this would cause no or hardly any perceptible change in the similarity). Dendrograms show well the differences of metal toxicity. These results proved to be similar to the experiments on growth, (see Fig. 1 - 4) as of the four heavy metals Cr has been found to be the most toxic both to the growth and to the element content, followed by Cd, Ni and Pb.

In summarizing the main results, this study have shown that heavy metals differently affect the essential micro element content of wheat seedlings. The changing of element proportion and quantity have shown to be probably sensitive parameters to indicate the toxicity of different heavy metals. Further investigation of the relationship of heavy metal toxicity on micro element content of plants may bring more interesting results.

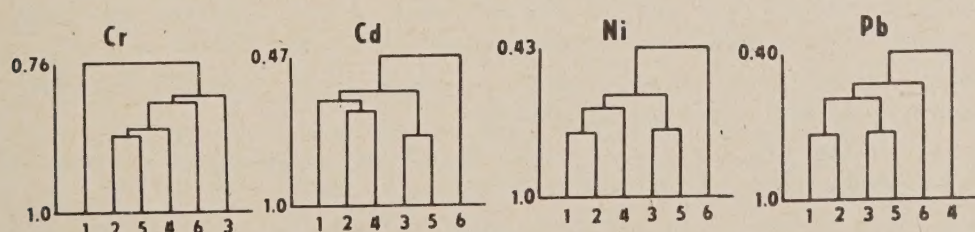


Fig. 5. Dendrograms of the heavy metal treatments. (1 = control, 2 = 10^{-5} M, 3 = $5 \cdot 10^{-5}$ M, 4 = 10^{-4} M, 5 = $5 \cdot 10^{-4}$ M, 6 = 10^{-3} M).

Tab. 2. Essential micro elements of wheat shoots changed by heavy metal treatment.

Concentration of heavy metals in the nutrient solution (M)	(µg/g dry weight)					
	B	Zn	Mn	Mo	Cu	Fe
0	10.90	31.25	42.31	41.94	10.52	62.42
10 ⁻⁵	10.89	27.11	34.22	84.77	8.06	63.25
5 . 10 ⁻⁵	11.51	31.62	35.46	96.82	9.33	71.66
Cd 10 ⁻⁴	9.70	35.75	40.68	62.74	9.12	83.13
5 . 10 ⁻⁴	5.58	41.57	31.87	11.70	9.43	79.64
10 ⁻³	3.29	67.21	40.36	8.50	15.20	108.67
10 ⁻⁵	9.49	21.59	37.64	37.78	9.92	40.66
5 . 10 ⁻⁵	10.95	25.93	33.39	17.75	8.49	50.90
Cr 10 ⁻⁴	8.62	24.61	27.82	3.66	7.18	52.66
5 . 10 ⁻⁴	7.79	33.56	29.05	2.65	8.98	63.53
10 ⁻³	11.25	32.49	37.48	3.93	9.91	69.55
10 ⁻⁵	2.26	39.04	27.24	12.95	7.93	69.32
5 . 10 ⁻⁵	1.65	26.29	25.88	10.58	7.74	60.88
Ni 10 ⁻⁴	1.12	24.51	20.03	9.16	7.20	52.54
5 . 10 ⁻⁴	1.44	68.12	25.29	9.10	9.97	67.56
10 ⁻³	1.60	136.10	31.41	8.34	11.79	98.96
10 ⁻⁵	7.19	25.16	36.81	52.70	11.38	61.37
5 . 10 ⁻⁵	7.07	27.50	40.71	38.07	12.64	59.71
Pb 10 ⁻⁴	9.07	27.74	40.50	24.97	11.79	58.40
5 . 10 ⁻⁴	7.76	30.76	29.77	11.91	10.90	66.26
10 ⁻³	10.97	37.84	35.75	5.69	9.49	72.64

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Biological monitoring in deteriorated Sokolov district

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I N T R O D U C T I O N

Thermal power plant stations are very serious sources of environmental pollution in our country. Many trace elements are concentrated in fly ash, slag, or fuel gases after the combustion, and are dispersed into the atmosphere or become waste disposal problems having a potential environmental and human health impact. Such a relatively higher accumulation of toxic substances in the ecosystems represents also a potential danger for landscape. In Sokolov district, there are also large dumps from open-cast mining. There exist changes in the species composition, participation of various forms of life ... Soil moisture and organic content are two most important factors in determining the establishment of vegetation species on mined land (primary production). Thus the spontaneous succession is followed there. In such deteriorated area we cannot investigate only the monitoring of function, structure and stability in started re-establishment ecosystem, but at the first stage of biological monitoring must be conditioned by the stable cycles of substances, all nutrients and also by ecotoxicologically significant microelements and flows of energies (CAIRNS, 1979; 1981). The situation in surface coal mines and other mined lands provides an opportunity to study the role of natural succession on the factors affecting soil characteristics, soil micro-organisms and the diversity and productivity of plant communities (BRENNER, 1985). The observations of the chemism of pollutants give a good opportunity for the determination of loading on these deteriorated ecosystems.

Therefore we have collected such species which can be found as biological entities present in all exposed stations. We have used direct bioindication - bioaccumulation, e. g. the determination of the concentration of a few ecotoxicologically interesting elements in adapted organisms without being damaged (KOVACS, 1986). Therefore, this method necessitates long field observation, some extensive experience and many experiments in laboratory.

S T U D Y A R E A

Sokolov district is the most deteriorated area in Bohemia: 1. mining activities; 2. coal combustion processes.

The total mined area is 70 km² and it creates about 10 % of the total land area from this district. Surface mine spoil consist of tuffs fragments, tuffites, impure coal, clay, dark kaolin, gravels and sandstone (KUBIZŇÁKOVÁ, 1987).

M E T H O D S

During 1986-87 years we have collected all samples in correspondence with the requirements of trace analysis. The concentrations of Al, Be, Cd, Cu, Pb, Tl, Zn and V were determined by AAS method on triplicate samples obtained from no more than 16 representative sites of this area. Surface water pH was determined by a pH-meter equipped with combined electrode. All samples were decomposed by wet pressure digestion in PTFE (in conc. HNO₃), after drying at 105° C.

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Tab. 1. The average concentration of some elements in various plants in surroundings of power plant station Tisova. x-mean value, s-standard deviation.

Plant	Number of samples	concentration in $\mu\text{g} \cdot \text{g}^{-1}$ dry mass									
		Cd		Cu		Ni		Pb		Zn	
		x	s	x	s	x	s	x	s	x	s
birch <i>Betula verrucosa</i>	8	0.32	0.09	8.9	6.3	8.4	3.3	2.6	0.6	129.0	60.0
rosebay willowherb <i>Chamaeneorion angustifolium</i>	14	0.08	0.02	10.0	3.8	7.4	7.3	1.5	8.5	40.4	15.4
bush vetch <i>Vicia cracca</i>	16	0.12	0.04	7.2	3.1	5.7	4.4	1.4	0.7	51.5	18.4
clover <i>Trifolium</i> sp.	16	0.09	0.07	10.2	3.1	5.1	2.8	3.9	3.8	50.8	9.1
common horsetail <i>Equisetum arvense</i>	16	0.25	0.37	6.5	1.5	4.2	1.8	2.0	0.4	36.8	13.9
common lady's <i>Alchemilla</i> sp.	10	0.36	0.27	7.5	2.5	5.4	3.7	2.3	0.7	60.9	13.3
coltfoot <i>Tussilago farfara</i>	14	0.11	0.07	6.6	2.5	10.7	12.5	2.7	1.5	42.1	12.5
yarrow <i>Achillea millef.</i>	16	0.37	0.32	10.3	0.75	5.9	4.5	2.5	1.9	36.3	23.2
soft rush <i>Juncus effusus</i>	4	0.15	0.09	4.9	1.6	6.6	5.4	1.0	-	27.7	8.9
lesser reedmace <i>Thypha angustifolia</i>	6	0.17	0.05	6.2	1.3	5.5	3.8	1.0	-	24.2	8.1
alder <i>Alnus glutinosa</i>	14	0.11	0.05	9.3	6.7	6.5	2.4	2.2	0.4	50.0	10.8
tree fungus <i>Fomes fomentarius</i>	3	17.60	3.60	115	12.9	-	-	50.0	10.9	4772	150
lichens <i>Cladonia</i>	5	0.05	-	9.9	2.8	3.0	1.1	10.9	3.1	26.9	11.3
moss <i>Fontinalis antipyretica</i>	6	3.2	2.1	46.7	13.5	192.8	18.7	35.3	8.9	1000	-
algae	5	1.2	0.5	53.2	13.8	136.3	15.4	3.2	1.2	1000	-

Tab. 2. The average concentration of some elements in various animals in surroundings of power plant station Tisova.

Animals	Concentration in $\mu\text{g} \cdot \text{g}^{-1}$ dry mass						Sampling location
	Al	Be	Cd	Cu	Pb	Zn	
<i>Lubricus terrestris</i>	2955	0.6	2.1	58.9	7.8	277.8	Hory
<i>Lubricus terrestris</i>	5643	0.5	5.7	34.9	101.5	1506.4	Alberov
<i>Lithobius forficatus</i>	255.6	0.5	3.8	95.8	1.1	108.2	Studenec
<i>Lithobius forficatus</i>	-	0.9	3.6	49.1	17.9	366.2	Alberov
<i>Lithobius forficatus</i>	93.3	0.6	3.7	152.1	6.2	487.5	Rajec
<i>Pterostichus</i> sp.	85.5	0.2	0.9	22.1	15.7	168.2	Alberov
<i>Pterostichus</i> sp.	156.3	0.4	2.3	33.2	7.8	214.8	Rajec
<i>Coelotes terrestris</i>	0	0.1	26.2	86.6	0	519.6	Alberov
<i>Coelotes terrestris</i>	67.7	0.2	0.5	44.6	3.4	188.6	Rajec
<i>Chorthippus</i> sp.	113.5	0.1	0.5	35.6	6.1	148.3	Lipnice
<i>Coccinella septempunctata</i>	203.5	2.7	4.1	54.4	27.1	474.8	Alberov
<i>Satyridae</i> sp. div.	248.8	0.5	2.5	34.8	0	781.2	Lipnice
<i>Oesticus verrucivorus</i>	118.1	0.1	0.3	27.6	0	154.7	Lipnice
<i>Hymenoptera</i> sp. div.	300.1	1.0	2.6	25.0	30.0	340.1	Lipnice
<i>Placycleis denticulasa</i>	133.3	0.3	0.3	20.8	0	156.7	Lipnice
<i>Zygaena</i> sp.	101.1	0.2	0.5	26.9	0.2	257.6	Lipnice
<i>Bufo viridis</i>	118.6	0.1	0.2	0.7	0.5	96.2	Alberov

Tab. 3. Soluble metal concentrations (in $\mu\text{g} \cdot \text{l}^{-1}$) in filtrated surface water in Sokolov district. Concentrations of Be are in KUBIZŇÁKOVÁ (1988).

No. Water	Sampling Location	C o n c e n t r a t i o n				pH	Remarks
		Al	Cu	Pb	Zn		
1	Družba	50	5	5	5	8.0	dump
2	Jiči	120	5	5	0	7.6	dump
3	Lipnice	1500	5	10	40	5.1	mine w.
4	Lipnice	1300	50	0	1000	5.5	mine w.
5	Vintířov	125	2	15	23	7.6	brook
6	Bedřich Anna	50	5	0	20	8.4	dump
7	Jiči	100	5	10	15	7.8	drainage
8	Družba	50	5	460	760	-	retence
9	Josef	45	1	10	-	5.8	mine w.
10	Týn	700	5	5	1280	-	dump
11	Vintířov	2400	10	0	340	-	drainage
13	Nové Sedlo	80	15	10	115	-	brook
14	Jimlikov	50	5	10	35	7.8	stream
15	Chodov	100	10	0	25	6.7	stream
16	Vlčí Vrch	100	2	13	15	7.2	pond

Tab. 4. Metal concentration in Sokolov District was calculated from average fallout (during 1975 - 1984 years by Hygienic Service Sokolov). Total average fallout was estimated about $230 \text{ t} \cdot \text{km}^{-2} \cdot \text{yr}^{-1}$. MIC maximum permissible concentration for open air (BENCKO, 1984). + as oxid (CdO , CuO , ZnO).

Element	Fallout	MIC ($\mu\text{g} \cdot \text{m}^{-3}$)
aluminium - Al	$9.3 \text{ t} \cdot \text{km}^{-2} \cdot \text{yr}^{-1}$	-
beryllium - Be	$1.1 \text{ kg} \cdot \text{km}^{-2} \cdot \text{yr}^{-1}$	0.01
cadmium - Cd	$0.34 \text{ kg} \cdot \text{km}^{-2} \cdot \text{yr}^{-1}$	3.00^{+}
copper - Cu	$30.9 \text{ kg} \cdot \text{km}^{-2} \cdot \text{yr}^{-1}$	2.00^{+}
lead - Pb	$25.7 \text{ kg} \cdot \text{km}^{-2} \cdot \text{yr}^{-1}$	0.70
zinc - Zn	$155.0 \text{ kg} \cdot \text{km}^{-2} \cdot \text{yr}^{-1}$	50.00^{+}

RESULTS AND DISCUSSION

In this paper the contents of Al, Be, Cd, Cu, Pb and Zn were determined in the chain: fallout - soil - surface water - various plants and terrestrial invertebrates and insects. The results of thallium and vanadium proved to be under detection limit. A few investigations showed the transfer of some elements into living organisms (Tab. 1, 2). For example, lichens are the bioaccumulator of Pb, moss (*Fontinalis antipyretica*) and algae can be applied to indicate Ni, Cu, Zn, Cd and also Be (KUBIZŇÁKOVÁ, 1988). Tree fungus can be considered as the bioaccumulator of Cd, Cu, Pb, Zn and Be.

We published some information on the risk factors of various toxic elements from burning Czech brown coal (KUBIZŇÁKOVÁ, 1987). It is as follows:

$\text{S} \gg \text{Cu} > \text{Be} > \text{Cr} > \text{Ni} > \text{Pb} > \text{Mn} > \text{Co} > \text{Zn} > \text{V} > \text{Se} > \text{As} > \text{Hg} > \text{Tl} > \text{Cd}$

The plants represent an essential link (Al, Cu, V, Zn) in the food chain, being the most important pathway for animals and human contamination (Be, Cd, Pb, Tl).

In Tab. 3 is shown the concentration of interesting elements in surface waters and also pH. It is clear that the mine waters are acidic ($\text{pH} \sim 5$) and dump waters are alkalic ($\text{pH} > 7$). The other waters depend not only on the origin, but also on the characteristics of catchment. The hydrology of Sokolov district is very hard to understand, because it is disturbed by mining activities. The situation is complicated also by acid rain and increase of wheathering processes in this area. It's possible that the local enrichment is due to exceeding geochemical cycle.

We calculated the annual fallout data collected by Hygienic Service from Sokolov during 1975 to 1984. This information can be used as the input of ecotoxicologically important elements into the polluted Sokolov district.

C O N C L U D I N G R E M A R K

At present, the commonly used methods of biological monitoring were not possible to use in Sokolov district. It is very hard to differentiate the effects of pollution and succession. For this reason the investigation is limited only to measuring the concentration levels of ecotoxicological elements. Chosen cumulative organisms will be accepted as indicators for further ecotoxicological research.

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Accumulative indicator plants on Hungarian spoils

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By the rapid expansion of areas of spoils of industrial and mining origin the danger is ever increasing that through the beasts feeding in such regions toxic elements uncontrollable get into the great biogeochemical cycles. First of all the amounts of elements accumulated in plants have to be determined to estimate the degree of this danger. The knowledge of such indicative values is also important to decide, whether the vegetation of the individual spoils is suitable for feeding domestic animals or possibly for human consumption. In order to answer these questions, the chemical composition of numerous plants settled down on four Hungarian spoils through a natural way were examined.

The main chemical characteristics of the spoils can be seen in Tab. 1. The investigations, which spanned 26 elements, were carried out after acidic exploration by atomic absorption spectrophotometry.

On the basis of the element content of plants it was established, that the amount of toxic elements present in the leaves is even at high pH-values greatly dependent on the chemical composition of the spoil. While in the case of the major nutritive elements - such as Ca, K, Mg and P - the element accumulation was primarily determined by the taxonomic status or the growth form of the species (Fig. 1.), the presence in the plants of the toxic aluminium, copper, zinc as well as iron was first of all affected by the habitat (Fig. 2.). On the spoils in Ajka, Gyöngyösoroszi and Borsodnádásd, at pH-values around 8.0, the leaves of several plants

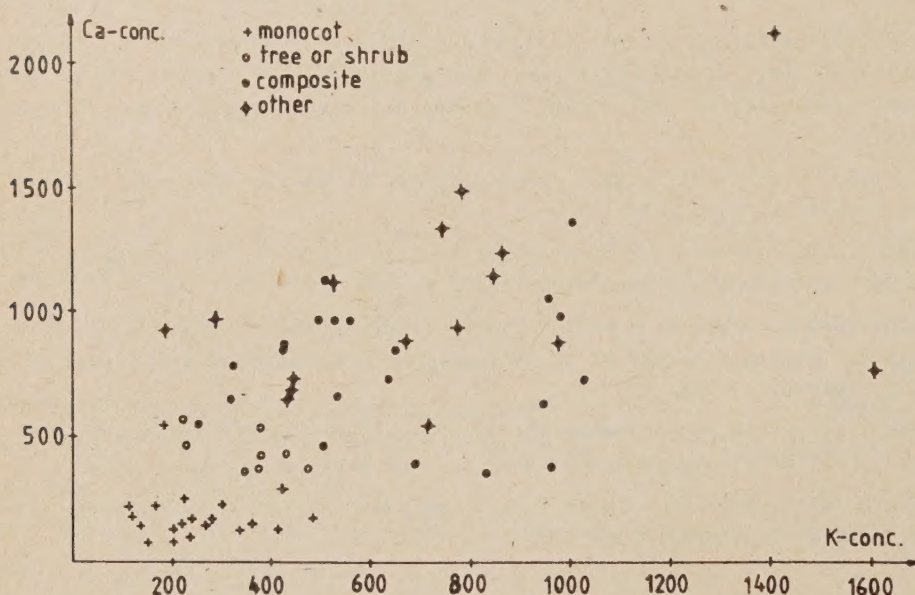


Fig. 1. Ca- versus K-concentrations in the leaves of plants of different taxonomic status or growth form, grown on spoils (concentrations in $\text{mmol} \cdot \text{kg dry weight}^{-1}$).

Tab. 1. The main chemical characteristics of spoils examined

Spoils	Elemets in excess	pH
Ajka	Al, Ca, Fe, Ti, V	above 8.0
Recsk	Cu, Fe	around 5.0
Gyöngyösoroszi	As, Co, Cu, Fe, Pb, Zn	7.5-8.5, but in some spots around 3.0
Borsodnádasd	Ca, Cd, Cr, Cu, Fe, Ga, Ni, Pb, Zn	above 8.0
Control		6.4

contained high amounts of aluminium, zinc and iron, respectively. It remains unclear, however, whether this element accumulation is due to an intake of nutritives or is the consequence of the tight adsorption of "unwashable" particles to the leaf surface. It is also considered as possible, that the thin acidic layer on the surface of the rootlets may have a part in the intake of the toxic alements (MENGEL, 1984).

Unexpectedly, of the 23 elements suitable for an appraise, in the case of 12, "oligotrophic" monocots accumulated the highest amounts. Especially *Puccinellia distans* is conspicuous among the investigated species with its high Al-, Co-, Cr-, Fe-, Ni-, P-, Ti- and V-content (Tab. 2.). Dividing the values of absolute element content by the respective element concentrations of the soil, relative accumulation values are obtained. Considering these ralative numbers, the monocots already do not have similar outstanding positions as mentioned before. The highest absolute element accumulation values in the leaves of plants can be seen in Tab. 2.

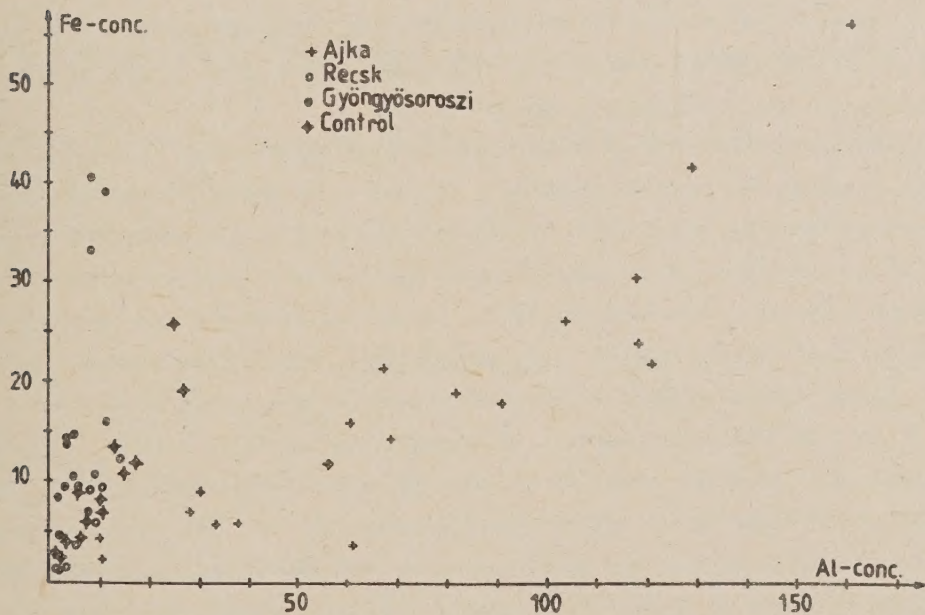


Fig. 2. Fe- versus Al-concentrations in the leaves of plants grown on different spoils (concentrations in mmol . kg dry weight⁻¹).

Tab. 2. Highest element concentrations in the leaves of plants grown on spoils (mmol . kg dry weight⁻¹).

Element	Concentration	Plant species	Habitat
Al	162.6	<i>Puccinellia distans</i>	Ajka
As	.1443	<i>Reseda lutea</i>	Gyöngyöskereszi
B	40.11	<i>Echium vulgare</i>	Ajka
Ba	.9553	<i>Daucus carota</i>	Control
Ca	2120	<i>Salsola kali</i>	Ajka
Cd	.0956	<i>Tussilago farfara</i>	Gyöngyöskereszi
Co	.0146	<i>Puccinellia distans</i>	Control
Cr	.0930	<i>Puccinellia distans</i>	Ajka
Cu	.8112	<i>Agrostis alba</i>	Gyöngyöskereszi
Fe	56.45	<i>Puccinellia distans</i>	Ajka
K	1624	<i>Salsola kali</i>	Control
Li	28.36	<i>Agrostis alba</i>	Ajka
Mg	767.0	<i>Erucastrum nastirciifolium</i>	Borsodnádásd
Mn	13.60	<i>Calamagrostis epigeios</i>	Recsk
Mo	1.231	<i>Reseda lutea</i>	Ajka
Na	298.2	<i>Sonchus arvensis</i>	Control
Ni	.1045	<i>Puccinellia distans</i>	Ajka
P	159.2	<i>Puccinellia distans</i>	Control
Pb	.6057	<i>Reseda lutea</i>	Gyöngyöskereszi
Si	38.17	<i>Calamagrostis epigeios</i>	Recsk
Sr	2.431	<i>Salsola kali</i>	Ajka
Ti	2.257	<i>Puccinellia distans</i>	Ajka
V	.4468	<i>Puccinellia distans</i>	Ajka
Zn	18.49	<i>Tussilago farfara</i>	Gyöngyöskereszi

Of Tab. 2 it can be seen, that the plants grown on spoils are rather poor in the main nutritive elements (e. g. K, P). It is also conspicuous, that *Puccinellia distans* is a very effective accumulator of several toxic elements. Our data also suggest, that *Daucus carota* is just as good accumulator of Ba, as is *Salsola kali* of K.

It was revealed that the ratio of the chemical components of plants grown on spoils significantly differs from that of the controls (Tab. 3.). In some cases the aluminium and iron content of the leaves exceeded by far their P- or (only in the case of Al) even their Mg-concentration. It is also uncommon, that leaves contain much more Mn than Fe (as those of *Calamagrostis epigeios* in Recsk), much more Zn than B (as the leaves of the same species in Gyöngyöskereszi) and 2.5 times more B than Fe (*Tussilago farfara*, Ajka). Tab. 3. shows that plants grown on the spoil in Ajka accumulate very high amounts of B and Fe, whereas their Mn-content is rather low, the leaves of plants from Gyöngyöskereszi have in contrast an excess of Cu, Fe and Zn, those from Recsk an excess of Mn, whereas those from Borsodnádásd are rich in Fe. The difference between the Mn-content of plants originating from Ajka and Recsk can be attributed to the difference between the pH-values of the two spoils (KINZEL, 1982). It seems, that the uptake by plants of Mn present in spoils in smaller quantities is more hindered by high pH-values, than the uptake of other elements, occurring in greater amounts (e. g. Al, Fe). It is worth considering, what kind of effect may have the found ratios of elements, divergent from that regarded as normal, on the further members of the food chains.

Tab. 3. Order of magnitude of element concentration in plants grown on different spoils
(mmol . kg dry weight⁻¹)

		P	Fe	B	Mn	Zn	Cu	Pb
Considered as normal (ERNST, 1982)		10 ²	10 ¹	5x10 ⁰	10 ⁰	10 ⁻¹	10 ⁻²	
Calamagrostis epigeios	Ajka, 1986	13.78	17.57	3.592	.2417	.3730	.0817	.0210
	Recsk, 1986	73.78	2.419	.4671	13.60	.5406	.0702	-
	Gyöngyöskereszi, 1986	32.61	14.45	.3159	1.977	4.895	.2954	.1390
	Control, 1986	36.87	8.906	.8857	1.496	.3596	.0591	.0205
Daucus carota	Ajka, 1986	34.00	12.21	6.763	.2727	.3788	.1193	.0246
	Recsk, 1986	85.79	8.646	3.920	2.077	1.190	.1924	.0208
	Control, 1986	77.17	10.74	4.202	1.639	.6056	.1299	-
Tussilago farfara	Ajka, 1986	52.92	8.714	21.21	.2084	.3832	.0997	-
	Recsk, 1986	48.47	2.620	5.937	.7099	1.255	.0922	.0242
	Gyöngyöskereszi, 1987	26.26	32.87	6.388	3.202	18.49	.5421	.0348
	Borsodnádásd, 1987	46.53	27.84	2.516	2.008	1.441	.1149	.0150
	Control, 1986	68.00	6.278	8.672	.4567	.6729	.1069	.0223

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Heavy metals in spruce needles and soils in polymetallic ore district of Zlaté hory (Czechoslovakia)

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INTRODUCTION

The biogeochemical sampling of vegetation and soil sampling are techniques used for prospecting of polymetallic mineral deposits. At the same time, they provide suitable methods for study of heavy metals migration in landscape contaminated by historical and recent mining activity and ore dressing. Application of biogeochemical sampling of plants for monitoring of the environmental impact of ore mining is substantially enhanced by statistical multispace analysis of data.

SAMPLING AREA

The Zlaté Hory ore district is situated in Northern Moravia in the northernmost part of a Devonian belt which forms a mantle of the crystalline core of the Hrubý Jeseník Mountains (SVOBODA et al., 1961; SUK et al., 1984). Disseminated polymetallic mineralization is formed mainly by pyrite, pyrrhotite, chalcopyrite, galena and sphalerite. Ore bodies of irregular shape and indistinct boundaries are situated in overthrust anticlinal structure which axis runs in a NW - SE direction. The Devonian volcanic-sedimentary complex is metamorphosed in a greenschist facies grade and consists of intercalated quartzites, chlorite-sericite schists, greenschists, metamorphosed quartz-keratophyres and their tuffs.

Landscape of the mining district territory is hilly and vegetation cover is formed by forest composed mostly of Norway spruce - *Picea abies* (L.) Karsten. Two phytocenological associations distributed in mosaic pattern were distinguished:

1. Calamagrostio-villosae-Piceetum (TUXEN 1937) F. K. Hartmann 1953 and 2. Vaccinio-myrtilli-Piceetum (SZAF., PAWL. et KULCZ. 1923) SOFRON 1981.

MATERIAL AND METHODS

Two biogeochemical profiles were situated at the mineral deposit Zlaté Hory - West with the main profile running across the main anticlinal ore-bearing structure and second one crossing perpendicularly the main profile in its middle part. The technique used for sampling is usual in biogeochemical prospecting of ore deposits which can be regarded as a special case of bio-indication.

At both profiles soils and twigs of Norway spruce - *Picea abies* (L.) Karsten were sampled. Distance of sampling points were 50 m. Soil samples were taken from the uppermost (A) horizon, dried, ground in a vibration mill and analyzed by energy-dispersive X-ray fluorescence spectrometry. Concentrations of sulphate ions were determined in distilled water leachates by V. Dombek using isotachopheresis. Spruce needles were dried, decomposed in a mixture of hot nitric acid with perchloric acid and analyzed by atomic absorption spectrophotometry.

Mathematical processing of data was performed by set of computer programs developed by MACHEK (1988). Methods of statistical multispace analysis - cluster analysis and principal components analysis were applied in order to attain better characterization of metal behaviour in soil and plants.

The main profile crosses the area of old mining works with collapses mining galleries from mediaeval times and old dumps of spoil rocks, then passes through the undisturbed area. The concentrations of most important ore elements (Cu, Pb, Zn) in the short interval at the middle part of the main profile as well as along the second profile are corresponding to the geochemical background. The main profile then passes through the geochemical anomaly where ore bodies are situated in the shallow depth of 30 - 80 m with surrounding lithochemical aureole reaching the surface.

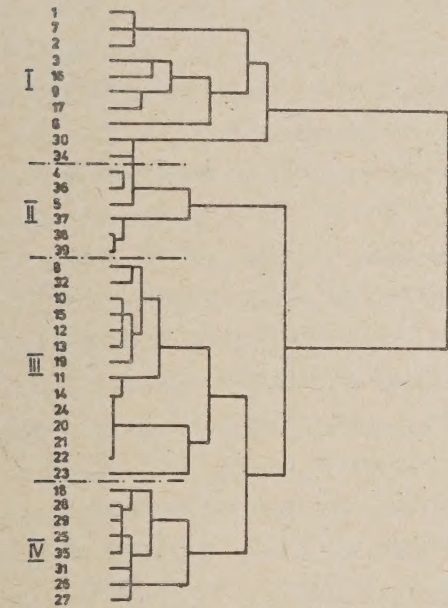


Fig. 1. Cluster analysis of soil samples from main profile.

RESULTS AND DISCUSSION

Example of cluster analysis for soil samples from main profile is represented in Fig. 1. Soil samples are divided into four basic clusters which differ one from another by variability of one or more chemical elements. Samples belonging to the cluster I have almost in all cases higher concentration of chemical elements, particularly contents of Pb and Zn are anomalous. Principal components analysis in Fig. 2 allows further characterization of considerable variability of chemism in the cluster I, which samples are scattered in a graphical plot through out the

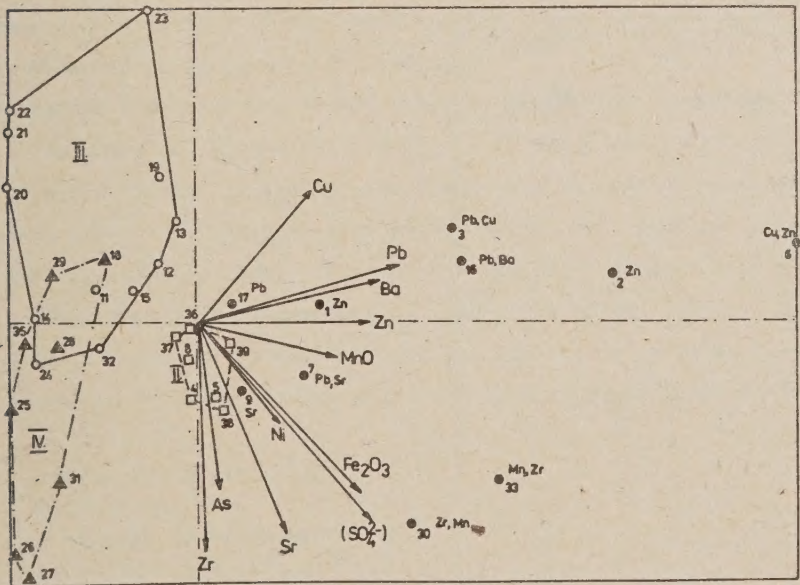


Fig. 2. Principal components analysis of soil samples from main profile (components 1 and 2).

wide area. The concentration of projection points for the cluster II into small area is determined by little variability of As and Ni. The cluster II differs from the cluster III and IV by higher concentrations of sulphate anions and by higher contents of Mn and Ni. Between the cluster II and IV there are significant differences in concentrations of Zr which has the main influence on the position of the cluster IV. The cluster III has also increased concentrations of Cu and Zn which allow to separate this cluster also graphically in the plot for principal components 1 and 3. Anomalous samples can be distinguished easily from principal

components analysis (Fig. 2): for Pb samples 3, 16 and 7, for Zn samples 1 and 2, for Cu samples 3 and 6, for Fe samples 30, 33, 7 and 9.

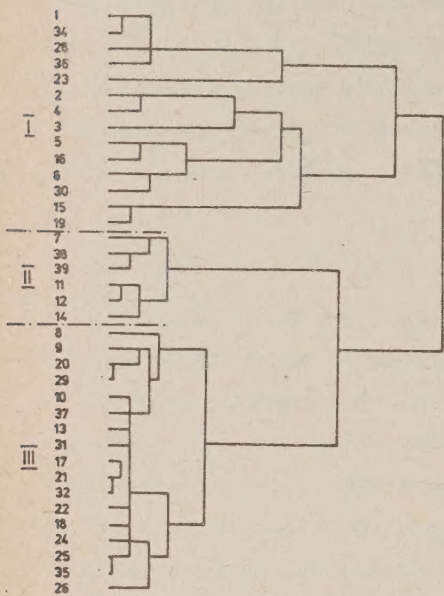


Fig. 3. Cluster analysis of biogeochemical samples from the main profile.

Cluster analysis and principal components analysis confirm the distinguishing of the geochemical background which was estimated by sampling an area without mineralization on second profile. Arithmetical means of metal concentrations from the main profile (prevalently increased or anomalous metal concentrations in soils) can be compared with those from geochemical background area (14 samples of second profile). All values are given in ppm except for Fe which is expressed as total iron content in %, the value of geochemical background is followed by the mean value of geochemical anomaly in parenthesis: Cu (132), Pb 50 (364), Zn 71 (334), Fe % 1.11 (2.13), Mn 335 (578), As 14.7 (54.1), Zr 180 (252), Ni 18.6 (29.8), Sr 44 (251), $(SO_4)^{-2}$ anomaly 131.

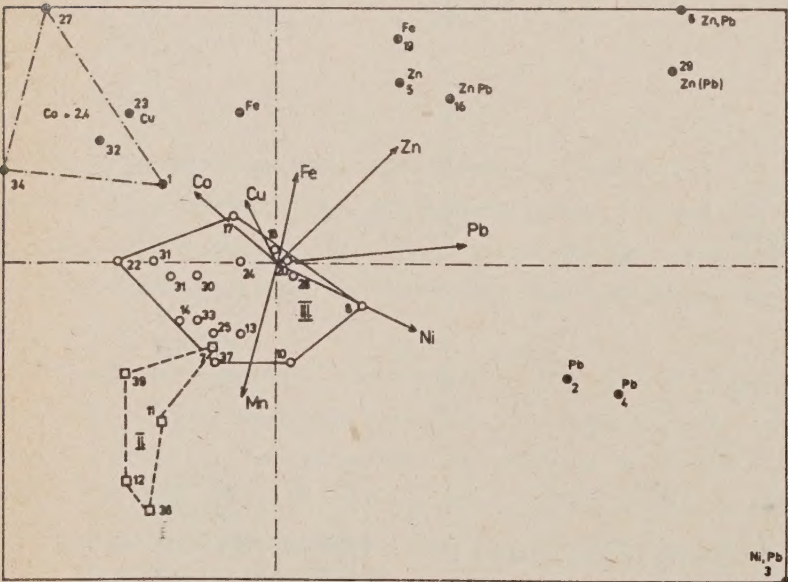


Fig. 4. Principal components analysis of biogeochemical samples from the main profile (components 1 and 2).

Similarly, values of arithmetical means of metal concentrations in biogeochemical samples (spruce needles) can be compared for the area of geochemical background and anomalous area. All values are given in ppm, mean anomalous values are in parenthesis: Cu 3.0 (11.7), Pb 2.15 (4.26) Zn 39.7 (38.1), Fe 175 (335), Mn 279 (432), Co 1 (1.2), Ni 1 (4.0). Data representing geochemical are in good accordance with literature (RÖSLER, LANGE, 1972).

Cluster analysis of biogeochemical samples from the main profile distinguished three main clusters which are graphically represented in Fig. 3. Cluster I is characterized by anomalous values of Pb (samples 2, 3, 4 and 6) and by higher concentrations of Co (samples 1, 32, 29 and 34). Anomalous values of other elements can be distinguished in graphical plot of principal components (Fig. 4). The cluster II is relatively small and contains group of samples with increased of Mn (7, 11, 12, 14, 36 and 37). The important role of Mn in defining the cluster II is similar to the behaviour of this element in soil samples. The most samples belong to the cluster III which can be distinguished from the cluster II in spite of the similarities in chemical composition.

Additional informations on migration of chemical elements in landscape and their relationships can be obtained by comparing the results of cluster analysis and principal component analysis for corresponding series of soil and biogeochemical samples. At the main profile there were distinguished four clusters for soil samples and three clusters for biogeochemical samples. In both sets the cluster I is representing the group of anomalous samples. Samples from seven sampling sites belong to the cluster I for soil samples and the same time to the cluster I for biogeochemical samples. The clusters II of both sets have 50 % of identical samples. When the clusters III and IV of soil samples are grouped together, percentage of corresponding samples with cluster III of spruce needles is 70 %.

C O N C L U S I O N S

1. Results obtained by application of cluster analysis and principal component analysis for biogeochemical data processing show the powerful possibilities of these techniques unambiguous distinguishing of anomalous samples, defining geochemical background and finding the inhomogenities of geochemical field.
2. Spruce trees growing in the areas of geochemical background on the territory of Zlaté Hory, mining district contain concentrations of heavy metals corresponding to the literature values for areas without natural geochemical anomalies and not contaminated by ore mining activities. Soils from these areas of geochemical background situated inside the ore district will be suitable material for land reclamation - revegetation projects.
3. Heavy metals concentrations in needles of Norway spruce - Picea abies (L.) Karsten represent suitable quantitative values which can be used for mineral deposits prospecting as well as for monitoring of environmental pollution by polymetallic ore mining and ore dressing.

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Mg-immission effect on natural ecosystems

M. KALETA

Plants for processing magnesite ores in Slovakia have an important position in national economy. Concerning quality and quantity of the produced fire-proof ceramics they take an important position in the world. Layers of crystalline magnesites longer than 150 km in the Slovak ore-bearing mountains form the important raw-material basis for this industry. A negative accompanying phenomenon of such a kind of industry, there are solid emissions getting into the atmosphere, which, in spite of using of filters, escape in such amounts that they influence negatively all the components of the life surroundings.

We observe systematically the influence of magnesite immissions on natural ecosystems near two plants in the middle parts of the valley of Muráň. Changes of the soil and vegetation characteristics were investigated at fix areas, geodetically defined, at different distances from the emission sources during 1966 - 1987. The investigations were initiated already before production began in our largest newly built enterprise near Jelšava.

By beginning production in the plant, also the amount of soil immissions increased, manifesting by broadening of the area of the damaged region. In 1975, an isoline bordered the area of the solid immissions surmounting the hygienic standard an area of 14.8 km², in 1976 33.3 km² and in 1977 34.0 km². It is necessary to underline that the hygienic standard of this kind of emissions does not protect the vegetation, the negatively influenced area is, therefore, considerably larger. The immissions of magnesite plants were of the following chemical composition: MgO - 65-80 %, FeO₃ - 7.5 %, CaO - 2.2 %, Al₂O₃ - 0.5 %, SiO₂ - 0.6 %, and further compounds in smaller and trace amounts.

SOIL CHARACTERISTICS

The soils below the stands of alluvial meadows form a whole scale of wet meadows soil with an underground water level in different heights. Soils, which are inundated in spring and early summer have a gleyish horizon 60-80 cm thick. They are grown by stands of Caricetum. During the years before acting of immission the soil reaction oscillated between pH 6.4-6.7 in water and MgO content was 10 mg/100 g maximally. In 1978-1987 we found considerable increasing of the soil reaction reaching in an average values of 8.9-9.2 pH in H₂O.

The most frequent soils in the alluvium of the flower Muráň are inundated transiently in the spring months, during the year the level of underground water is 60-70 cm below the surface. The soil reaction oscillated before acting the immissions between 6.3 and 7.6 pH, MgO content reached maximally 25 mg/100 g. Systematic following of the soil reaction has shown that the stepwise increasing of the reaction values stops at pH 9.5 in H₂O. The MgO content increased gradually reaching an increase by 100 % in an average.

Soils of wet meadows posed the highest in relation to the underground water, are grown by associations of Arrhenatheretum elatioris. The soil reaction increased from the original values of 7.5 pH to 9.5 pH. A similar tendency of increasing soil reaction and increasing MgO

content was found at soil types in the whole influenced region included brown forest and rendzinas. In spite of that we investigated also further chemical features of the soils (CaCO_3 , CaO , K_2O , Na_2O , R_2O , C, N, humus), we did not find more important influences of the immissions moreover to MgO and pH.

Among the microbiological characteristics of the influenced soils we found considerable decrease of the cellulolytic activity and the absence of nitrophilous microorganisms.

VEGETATION RELATIONS

Stands of the association *Caricetum gracilis* grow in terrain depression of alluvial meadows; before influencing by immission, the number of plant species at an area of 16 m^2 oscillated between 20 and 29 at 100 % covering. The species *Carex gracilis*, *C. riparia*, *C. vulpina*, *C. panicea* dominated.

During the years in question, the vegetation structure changed in direct relation to the alkalization of the soil. At an equal area, gradually the number of species decreased to 4-8, the composition of the species changed as well, *Carex diastans* and *C. hirta* becoming dominant species. From the original species of the association occurred in lower number *Carex gracilis*, *Poa trivialis*, *Lychnis flos-cuculi*.

The association of alluvial meadow formed by stand of *Alopecuretum pratensis*, inhabitates the soils in the spring months only being temporarily overflowed. It is an association rich on species oscillating between 26-32 species at an area of 16 m^2 , with 100 % covering. Among the dominant species belong *Alopecurus pratensis*, *Sanguisorba officinalis*, *Caltha palustris*, *Myosotis palustris*, *Festuca pratensis* and others. Under the influence of the magnesite immission the number of species decreased step by step, the sensitive ones disappearing the first (*Myosotis palustris*, *Cardamine pratensis*, *Caltha palustris*); relatively resistant species remained (*Festuca pratensis*, *Poa pratensis*, *Poa trivialis*). At the final degradation stage of the original association dominated the species *Agropyron repens*, *Puccinellia distans*, *Convolvulus arvensis*, *Saponaria officinalis*, *Silene inflata*.

The association the richest on species, is formed by stands of the association *Arrhenatheretum elatioris*, the average number at an area of 16 m^2 there was 35. Characteristical species of the association are formed by *Campanula patula*, *Crepis biennis*, *Knautia arvensis*, *Pastinaca sativa*, *Arrhenatherum elatius*, *Gallium molugo*. After more years influencing, the number of the species in the association decreased and transient stages of the vegetation were formed by relatively resistant species (*Dactylis glomerata*, *Poa pratensis*); the final degradation phase formed in the course of 20 years, is formed by the species *Agropyron repens*, *Falcaria vulgaris*, *Puccinellia distans*, *Convolvulus arvensis*.

The meadows are represented by stand of the association *Lolio-Cynosuretum*, characteristical species being *Cynosurum cristatum*, *Phleum pratense*, *Lolium perenne*, *Trifolium repens*, *Carastium vulgare*, *Plantago lanceolata* and others, with a total average number of 30 species at an area of 16 m^2 . During our observations, the number of species decreased to 4-6 (*Puccinellia distans*, *Chenopodium glaucum*, *Sonchus arvensis*), similarly as at the former associations.

Termophilous limestone slopes are grown by stands of the association *Festucion*, significant by a high number of species (35-48 at 20 m^2), many of them being protected and rare. In most

cases occurred the species Potentilla arenaria, Alyssum montanum, Thymus praecox, Teucrium chamaedrys, Salvia pratensis, Erysimum pannonicum and further. After a more years influencing by immissions, the association degraded, from the original species there remained Jovibarba hirta, Potentilla arenaria, Alyssum montanum, Erysimum pannonicum. New species penetrated, as Puccinellia distans, Sonchus arvensis strange to the associations.

Forest association of the influenced region in the valley of Muráň are formed by stands of the association Cephalanthero-Fagetum, Querco-Carpinetum and Picetum. At all forest associations the influence of immissions manifested by stepwise dieing of the top parts of the crowns according to the resistance of the single species (the most sensitive Picea abies, Pinus sylvestris, middle sensitive Quercus petraea, Carpinus betulus, Fagus sylvatica, the species of the genus Acer, resistant Pinus nigra). Drying of parts of crown has a higher irradiation of the soil as a consequence together with invasive propagation of resistant species (Puccinellia distans, Sonchus arvensis, Calamagrostis epigejos).

Knowledge of the dynamics of degradation within the single plant association, of its single stages and of the final synantropous associations at the region of magnesite plants, characterises the situation of the ecosystems, the degree of the devastation of the region. Propagation of the single species (e.g. Puccinellia distans) indicates in time the influence of solid immissions, and, finally, stating of the original study of soil and vegetation before influencing by immissions serves as a basis for eventual melioration work in the future.

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The role of metals in the multistress disease killing forests in Europe

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The Department of Environmental Conservation, University of Helsinki has taken on the task of studying the role of metals in the multistress disease killing forests in Europe. This article summarizes the mostly unpublished results of a working group.

The work began with a comparison of the metal contents in the biota of the forested water catchment areas of an acidified and a non-acidified lake lying quite near each other in Espoo. Clearly, more metals were present in the sediments, plants and animals of the acidified lake. In the terrestrial plants of the water catchments, however, only slightly higher metal levels were noted in the area surrounding the acidic lake. The plant samples had been collected so that they represented the entire catchment areas.

The primary study was expanded to microhabitat level by studying the metal contents of Vaccinium myrtillus in different soil fertility types. When these levels were compared between the two water catchments, no differences were detected. It was concluded that the microhabitat assortment in the water catchment area determines the degree of lake acidification and, simultaneously, the metal levels. We also noted that the Fe and Al contents in V. myrtillus increased towards the lake shores.

When we compared the metal levels in V. myrtillus leaves in spring and autumn, we noted clearly higher autumn levels. Later we noted that this is true for most plant species and most metals.

Because our task was to note the role of metals in forest decline, we also compared the metal levels in some indicator plants in forests which showed different degrees of disease (crown thinning in conifers). For this purpose we selected a set of indicator plants, which form a series of decreasing root contact with mineral soil. Their background metal levels were tested in unpolluted Lapland. When we analyzed the metal levels in indicator plants growing in forests with different degrees of forest damage, we noted in Espoo no other differences than highly elevated Al and Fe contents in the most heavily damaged forest area. This anomaly was, however, an artefact caused by dust from a highway. When we studied the metal levels of indicator plants growing under given single trees showing different degrees of damage (in the parish Perniö), we noted no correlation between the metal contents and degree of damage, except for mercury and cadmium in the rootless moss Pleurozium schreberi.

In Finland soil tilling is a common measure in the regeneration of cleared forests. In the podsolized forest soils, the tillings raise the alkaline enrichment layer (and the metals in it) to the acid top of the soil. We wanted to see the effects of tilling on the metal contents in some indicator plants. We collected material from the tilled and untilled areas of the Parkano Forest Research Station 8 years after the tilling. Analyses performed on Mainthium bifolium, Vaccinium myrtillus and Betula spp. showed no drastic changes but the levels of Al and, to a lesser degree, Cd and Zn were elevated in birch and blueberry. We noted also that

BOHÁČ J., RŮŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deteriorisationis Regionis. Institute of Landscape Ecology CAS, České Budějovice, pp. 306-308.

there was slightly more insect damage on the leaves of birches growing in the tilled areas. The difference was statistically significant for bark gnawings but not for other types of damage.

Also the transfer of metals from plants and detritus to animals was studied. First, in the Espoo forests we analyzed the metal contents in several animal species belonging to different trophic levels of the food chains (of the food web). Astonishingly, there occurred no clear bioaccumulation, although it was possible to note considerable specific variations and peak contents in unexpected species. Other rules than bioaccumulation along food chains determine the distribution of metals in forest ecosystems. Higher metal levels were found in micromammals living in the water catchment area of the acidified lake than of the nonacidified lake, but this difference occurred unclearly in invertebrates.

Effective excretion seems to be the main factor inhibiting bioaccumulation in forest animals. We noted this for five species of moths, which had as adults lower levels of Al, Fe, Mn, Cu and Cd than occurred in their food plants. Zn, on the contrary, accumulated in the moths at elevated levels - possibly in order to eliminate the more toxic elements. Small pine feeding moths seemed to have higher metal contents than did large ones.

We found a "hot line" for transfer of high contents of cadmium: it occurred at high levels in the soil humus inhabiting earthworm, in cambium of conifers, in cambium feeding bark beetles (not in xylophagous) and in Formica - ants feeding on the honeydew of phloem-feeding cynipid aphids. Hypothetically, Cd accumulation in ants may decrease their effectiveness as forest pest predators and thus increase the pest stresses to forest trees.

We noted in the crassulacean plant Sedum telephium such high Cd contents that may be, toxic for the papilionid butterfly Parnassius apollo which feed on it. This butterfly is strongly declining in Finland. We performed a comparative study on the metal levels in S. telephium in areas where P. apollo had disappeared and in areas where it still exists. For cadmium, no differences existed between the two types of areas (2.6 and 2.7 ppm Cd respectively), but clearly more Fe and Al were present in the areas where P. apollo had disappeared (Fe 123 ± 13 and Al 44 ± 5 ppm) than in the areas where it still exists (Fe 79 ± 4 and Al 28 ± 3 ppm).

We tested the effects of Cd-spraying on birch leaves on the occurrence of herbivorous arthropods. The spraying elevated the Cd contents in treated plants 7, 10 and 1 000 times higher than in normal birch leaves. In spite of this, no significant increases were found in the populations of herbivorous insects and gall mites. The resistance of birch and its herbivorous arthropods to Cd is astonishingly high.

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Soil macrofauna and small mammals as animal indicators of the biological availability of heavy metals in terrestrial environment

WEI-CHUN MA

INTRODUCTION

Soil is an ultimate sink for heavy metals released into the environment by industrial and agricultural processes. In industrialized countries, soil contamination with heavy metals is a serious problem and the continuing accumulation of metals in the soil calls for legislative protective measures. From this point of view there is an urgent need for ecotoxicological studies to develop meaningful instruments for the protection of the soil environment.

Invertebrate and vertebrate soil animals can be of great importance as bioindicators of soil pollution, as the amounts of metals found in body tissues and also the toxic effects of metal exposure reflect the biologically available amounts present in the soil. The purpose of this paper is to demonstrate the use of soil macrofauna and small mammals as indicators of the bioavailability of heavy metals in polluted terrestrial environments. In my opinion, the results of these studies can be applied to differentiate present standards of soil pollution according to the availability to organisms. The data used in this paper are drawn from studies which I have carried out in The Netherlands (MA et al., 1983; EDELMAN et al., 1984; MA, 1987) and also include some unpublished results.

SITE DESCRIPTION AND METHODS

The study area was situated near Budel in the south of the Netherlands close to the border with Belgium (Fig. 1). This region, which has a sandy soil type and includes both agricultural fields and forests, has been influenced for several decades by emissions of cadmium (Cd), lead (Pb), and zinc (Zn) from a metal smelter and refinery located at Budel.

Soil surveys showed that the pollution of the surface soil with Cd, Pb, and Zn extended over a distance of several kilometers downwind to the smelter. The dispersal pattern of the pollution has been illustrated for Zn in Fig. 1. Similar patterns were found for Cd and Pb. I selected a site at a distance of about 1.5 to 2 km downwind from the smelter in order to study metal accumulation in soil, vegetation, macro-invertebrates and small mammals. Concentrations of Cd, Pb, and Zn in these samples were compared with those of samples collected from a reference site near Arnhem situated at about 125 km north to the smelter. Both study sites had a similar type of podzolic sandy soil. The choice of invertebrates and plants was based on the known relevance of the samples as food items for small mammals. Thus, above-ground parts of grasses and herbaceous plants were used which are eaten by field voles (FABER, MA, 1987), while the selected invertebrate samples were utilized as food by shrews as indicated by analysis of stomach contents. Metal contents were determined by sample destruction in concentrated nitric acid and measured with atomic absorption spectrometry and are all expressed in $\mu\text{g/g}$ dry mass.

Tab. 1. Concentration ($\mu\text{g/g}$ dry mass) of metals in soil, litter, vegetation, and soil invertebrates of two study sites. Mean values of 20 samples. Coleoptera excluding Carabidae, Lumbricidae with emptied gut contents.

Substrate	Smelter site			Reference site		
	Cd	Pb	Zn	Cd	Pb	Zn
Invertebrates						
Coleoptera	3.8 ± 0.5	19.0 ± 3.0	373 ± 36.9	1.3 ± 0.1	12.0 ± 2.0	203 ± 9.0
Carabidae	4.2 ± 0.9	11.9 ± 2.1	260 ± 28.3	1.9 ± 0.9	6.0 ± 1.3	149 ± 12.4
Araneae	51.8 ± 6.1	36.4 ± 8.1	1624 ± 92.4	17.2 ± 0.4	10.5 ± 1.3	827 ± 52.0
Opiliones	16.0 ± 1.9	18.0 ± 1.7	1502 ± 183	4.5 ± 1.2	17.3 ± 3.8	513 ± 46.5
Lumbricidae	91.8 ± 15.2	162 ± 37.2	2348 ± 306	27.1 ± 7.3	63.5 ± 9.9	456 ± 76.3
Vegetation						
Grasser	1.3 ± 0.2	8.6 ± 1.2	289 ± 26.1	0.5 ± 0.06	5.3 ± 1.0	70.7 ± 4.9
Herbs	1.1 ± 0.2	5.1 ± 0.7	105 ± 6.7	0.3 ± 0.05	6.6 ± 0.7	47.1 ± 3.3
Soil						
A ₀ -layer	5.7 ± 0.3	266 ± 67.6	1020 ± 81.0	1.2 ± 0.1	191 ± 8.7	110 ± 8.8
Mineral layer	2.9 ± 0.1	128 ± 15.9	301 ± 23.2	0.2 ± 0.05	21.5 ± 9.0	9.4 ± 2.9

METAL ACCUMULATION IN BIOTA

The average concentrations of Cd, Pb and Zn measured in the various samples of soil, litter, vegetation, and invertebrates are shown in Tab. 1. Of the soil macrofauna samples only the Lumbricid earthworms showed an elevated body content of all three metals at the smelter site. The spiders and beetles showed a significantly elevated concentration of Cd and Zn, but not of Pb. As shown in Tab. 1, the contamination of Pb present in the reference site did not differ from the contamination of the smelter site as to the presence in the A₀-layer, but did so

in the underlying mineral soil layer. The unexpected high Pb contamination of the litter layer at the reference site has probably been due to emissions from traffic or other air pollution sources near Arnhem. Apparently, the earthworms had accumulated the metals primarily from the deeper soil layers, whereas spiders and beetles are contaminated by metals present in the superficial litter layer of the soil. Another interesting feature which can be derived from Tab. 1 is the higher accumulation of Cd, Pb and Zn in the soil-exposed earthworms compared to the litter-exposed spiders and beetles, in spite of the fact that the soil had lower metal concentrations than the litter layer.

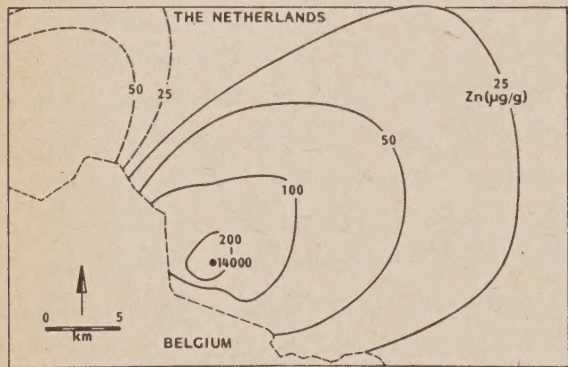


Fig. 1. Isolines showing the geographic dispersal pattern of the soil contamination with zinc near the smelter at Budel (black dot).

The order of decreasing metal accumulation in the various groups of soil macrofauna can be given as follows:

Lumbricidae > Araneae > Opiliones > Coleoptera

This order may be due to specific differences in accumulation, metabolism and/or excretion of the metals in these animals and/or to differences in the available form in which the metals occur in the food. It is important to note that lumbricid earthworms apparently provide a more important source of metal contamination to vertebrate predators of soil macrofauna than do the surface-living invertebrates. Plant-eating vertebrates are apparently less exposed to metal contamination than invertebrate predators because of the lower metal concentrations present on a dry weight basis in vegetation than in invertebrate samples. I shall come back to these points later on in this paper.

FACTORS AFFECTING THE ACCUMULATION IN EARTHWORMS

The potential of lumbricid earthworms to accumulate heavy metals has been investigated further for Lumbricus rubellus. This species was sampled from 31 different grassland and heathland locations scattered throughout the smelter area. The soil of the various locations differed widely in their organic matter content, i. e., from 2.2 to 8.6 %, as well as in pH (KCl) which varied between 3.5 and 6.1. Only adult specimens of earthworms with emptied gut contents were used for analysis.

Tab. 2 shows the wideness of the range in metal concentration among the earthworm samples. This wide range suggests that earthworms are able to tolerate high body burdens of Cd, Pb, and Zn, which is probably made possible by certain detoxification mechanisms such as the metal storage capability of the chloragogenous tissue.

The variation of the concentration values shown in Table 2 was analyzed statistically by multiple linear regression analysis using soil metal concentration and edaphic parameters as independent variables. Significant variables affecting the metal concentration in the earthworms are shown in Tab. 3. In each case, the variables together accounted for about 70 % (adjusted R-square) of the variation in earthworm metal concentration.

Soil pH appears as a significant factor influencing the bioaccumulation of Cd, Pb as well as of Zn in the earthworms, while the organic matter content played a significant additional role in the bioaccumulation of Pb. The role of organic matter content and acidity as significant edaphic factors affecting the metal accumulation in the earthworms can readily be explained from the influence of these factors on the metal retention capacity in sandy soils. It is clear from these results that no simple relationship exists between the total concentration of a metal element in the soil and the accumulation in organisms and that therefore pollution standards should be differentiated according to the bioavailable status of the pollution.

ACCUMULATION IN SMALL MAMMALS

Small mammals are an interesting group of animals with respect to their possible use in studying pollution effects in terrestrial vertebrates and in studying the behaviour of pollutants in terrestrial food chains. I shall discuss three species of small mammals, i. e., the common shrew (Sorex araneus) and the mole (Talpa europea) as representatives of predators, and the field vole (Microtus agrestis) as a typical herbivorous species.

Tab. 2. Concentration range ($\mu\text{g/g}$ dry mass) of metals in soil and *Lumbricus rubellus* in the smelter area. Background ranges for sandy soils found elsewhere in The Netherlands are also given.

Metal	Smelter area		Background range	
	Soil	<i>L. rubellus</i>	Soil	<i>L. rubellus</i>
Cd	0.1 - 5.7	18 - 202	0.1 - 0.7	6 - 26
Pb	14 - 430	9 - 670	3.1 - 43	9 - 21
Zn	10 - 1220	717 - 3500	6.4 - 62	439 - 671

Tab. 3. Relationships between metal concentration in *Lumbricus rubellus* and soil metal concentration ($\mu\text{g/g}$ dry mass), soil organic matter content (% OM), and pH (KCl).

ln conc. Cd worm = 5.54 + 0.66 (ln conc. Cd soil) - 0.40 (pH)
ln conc. Pb worm = 4.16 + 1.13 (ln conc. Pb soil) - 0.18 (% OM) - 0.75 (pH)
ln conc. Zn worm = 6.79 + 0.34 (ln conc. Zn soil) - 0.27 (pH)

Tab. 4. Tissue metal concentration ($\mu\text{g/g}$) in small mammals collected from the same study sites as shown in Tab. 2. Means of 75 animals.

	Smelter area			Reference area		
	Cd	Pb	Zn	Cd	Pb	Zn
<i>Microtus agrestis</i>						
Kidney	2.8 $\pm$ 0.2	4.4 $\pm$ 2.2	85 $\pm$ 0.8	0.2 $\pm$ 0.02	3.6 $\pm$ 0.2	80 $\pm$ 0.7
Liver	0.6 $\pm$ 0.1	1.2 $\pm$ 0.07	91 $\pm$ 0.8	0.1 $\pm$ 0.01	1.1 $\pm$ 0.06	85 $\pm$ 0.8
<i>Sorex araneus</i>						
Kidney	128 $\pm$ 11	85 $\pm$ 21	173 $\pm$ 8.6	34 $\pm$ 2.7	58 $\pm$ 7.9	105 $\pm$ 3.4
Liver	167 $\pm$ 15	4.7 $\pm$ 0.6	127 $\pm$ 3.0	30 $\pm$ 2.2	5.1 $\pm$ 0.4	98 $\pm$ 2.7

Tab. 4 shows the average concentration of Cd, Pb and Zn measured in the kidney and liver organs of year-through samples of field voles and shrews. It can be seen that the two species show an extremely large difference in metal accumulation, especially of Cd and Pb. Values measured in shrews were many times higher than in field voles. This suggests that shrews are at a greater risk in metal polluted environments than herbivorous species of small mammals assuming an equal intrinsic sensitivity to metal exposure. The difference in metal accumulation between shrews and field voles may be due to the difference in food contamination. As shown above in Tab. 1, plants accumulated less Cd, Pb and Zn than did the invertebrates.

Shrews collected from the smelter site showed an elevated tissue level of both Cd, Pb and Zn, compared to animals from the reference site. The field voles, however, only showed

Tab. 5. Concentration of Pb in soil, *Lumbricus rubellus*, and *Talpa europea* from two different biotopes at the smelter area and from a reference site. Means and ranges of five samples. Earth worms emptied gut contents.

Study site	% OM	pH (KCL)	Pb concentration (µg/g dw)		
			Soil	Earthworms	Mole kidney
Smelter site					
Crassland	10.2	6.5	135	20	18 (8 - 35)
Heathland	2.0	4.1	149	590	338 (238 - 438)
Reference site					
Grassland	5.7	4.0	24	41	9 (6 - 11)

a difference for Cd and Zn, but not for Pb, between the two sites. This again may be correlated with the contamination of the food. Thus, the vegetation of both sites contained similar levels of Pb, but differed with regard to Cd or Zn (Tab. 1). In earthworms, however, which are a main food item for shrews there was a significant difference in Pb concentration between the two areas.

BIOAVAILABILITY TO EARTHWORMS AND MOLES

If lumbricid earthworms are an important food item for shrews, this is even more true for moles. It can be expected that the contamination of earthworms should have a direct bearing on the body burden of these specialist predators. I have taken the soil-earthworm-mole relationship as a simple indicator model for the movement of heavy metals in terrestrial food chains. In the following example, earthworms and moles were collected from two different locations in the smelter area. The two locations consisted of a cultivated grassland and a *Deschampsia* heathland

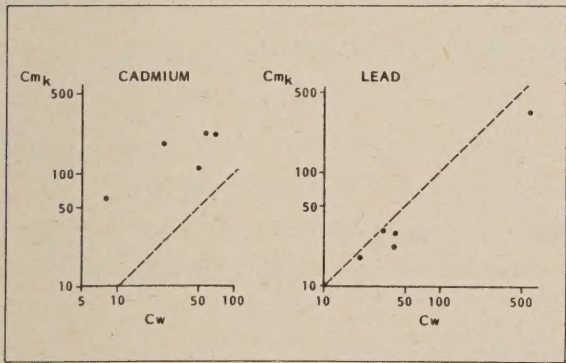


Fig. 2. Concentration of Cd and Pb in mole kidney in relation to the level in *Lumbricus rubellus* for five different biotopes. Each point is the average of five samples.

biotope. The soil of the two locations contained a contamination level of Pb, but it differed in organic matter content and pH (Tab. 5). A grassland biotope in the reference area near Arnhem was also investigated. In all three locations the accumulation of Pb was determined in earthworms, consisting of adult *L. rubellus* with emptied gut contents, and kidney of moles. The results of the measurements are shown also in Tab. 5. It can be seen that the bioaccumulation of Pb in the smelter area was much greater in the heathland location than in those from the grassland biotope. Thus, earthworms from the heathland biotope showed an almost 30 times higher Pb level than those of the grassland biotope, while the kidney of the moles showed an almost 20 times difference.

Some specimens of moles from the heathland even showed Pb values of more than 400 µg/g dry mass in kidney. By contrast, the kidney concentration of Pb of moles from the grassland biotope was hardly different if at all from the reference value.

The results shown in Tab. 5 indicate that Pb was hardly or not bioavailable in the limed grassland biotope, whereas it was highly so in the heathland biotope. The difference in bioavailability between the two sites can be attributed to the difference in soil organic matter content and pH, as suggested above in Tab. 3.

In a second study, the relationship between the availability of soil metal contamination to earthworms (*L. rubellus* with emptied gut) and the accumulation in moles was investigated. Samples of both organisms were collected in five different locations in the smelter area and reference area and measured for the accumulation of Pb and Cd.

The results are shown in Fig. 2. It appears that the concentration of these metals in earthworms (C_w) and in kidney of moles (C_{m_k}) shows a positive correlation. It can also be seen that the potential to accumulate in mole kidney is greater for Cd than for Pb. Thus, comparison with the 1 : 1 relationship shows that $C_{m_k}/C_w > 1$ for Cd, whereas $C_{m_k}/C_w < 1$ for Pb.

SUMMARY AND CONCLUSIONS

Summarizing it can be stated that soil macrofauna and small mammals are useful indicators of the bioavailable status of soil metal contamination in terrestrial environments. It is suggested that one should differentiate between soil-living lumbricids and surface-active macro-invertebrates such as spiders and beetles, because they are exposed to contamination present in different soil strata. The contamination of small mammals depends on their predatory or herbivorous feeding habit. Predatory small mammals such as shrews and moles are exposed to much higher contamination levels than herbivorous species such as field voles. The contamination of predatory small mammals is predominantly determined by the contamination present in lumbricid earthworms, which in turn is determined by the metal level, the organic matter content, and the acidity of the soil. Cadmium is shown to have a greater bioaccumulation potential than lead in terrestrial food chains. Finally, it is suggested that pollution standards should differentiate according to the bioavailability of the contaminants in soil to organisms.

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On the dynamics of cadmium and lead in *Steatoda bipunctata* (Araneae)

I. H. S. CLAUSEN

INTRODUCTION

This paper reports some of the results of a research projekt dealing with the behaviour of Pb, Cd, and Al in a foodchain system, focusing especially on Cd in the spider *Steatoda bipunctata*.

MATERIALS AND METHODS

Fruit-flies (*Drosophila hydei*) were reared in media containing different concentrations of lead and cadmium; Ppm Pb (of dryweight): 40 ppm (A), 200 ppm (B), and 1000 ppm (C); ppm Cd: 2 ppm (a), 7 ppm (b), and 24 ppm (c). Mature females and juveniles of *Steatoda bipunctata* were fed with different combinations of the metal-loaded *Drosophila*. The spiders were kept in plastic vials with a piece of moist filter-paper. At intervals the spiders were transferred to clean vials, and the remains of prey sampled for metal-analyses. After use the vials were washed with 1N HNO₃, and the nitric acid analysed for Cd. The experimental period was from 30.11.1987 to 19.02.1988 where the spiders were killed and digested in nitric acid and hydrogen-peroxide. Fruitflies and prey remains were digested likewise, and all metal-analyses were performed using atomic absorption spectrophotometry with graphite furnace. The results reported here concern the period in the last vial.

A significance-level of 0.05 has been chosen throughout.

RESULTS

No interactions between Cd and Pb were noted as far as the concentrations of the metals are concerned (According to two-factor ANOVA).

Correlations between Pb in *Drosophila* and media, and Pb in *Steatoda* and *Drosophila* were highly significant ($r_s = 0.933$, $P < 0.0005$, $n = 12$; $r_s = 0.793$, $P < 0.0005$, $N = 57$, respectively). Similar results were obtained for Cd in *Drosophila* and media, and Cd in *Steatoda* and *Drosophila* ($r_s = 0.827$, $P < 0.0005$, $N = 65$; $r_s = 0.888$, $P < 0.0005$, $N = 57$, respectively).

Pb in *Steatoda* reached an upper level of 8 ppm (7.3 - 9.3) (geometric mean, 95 % confidence interval, $N = 30$) at concentrations in *Drosophila* of about 5 ppm (Fig. 1).

Cd in *Steatoda* did not seem to reach an upper level under these experimental conditions (Fig. 2).

Drosophila did not biomagnify Pb, the biological concentration factors (ppm Pb in *Drosophila*/ppm Pb in medium) ranging from 0.07 to 0.16.

Steatoda seemed to biomagnify Pb in the control-group, and in the A and B groups, the biological concentration factors (BCF) (ppm Pb in *Steatoda*/ppm Pb in *Drosophila*) being 4.8, 3.0 and 2.0, respectively. However, in the C-group the BCF was 0.14. There was a significant negative correlation between the Pb-BCF of *Steatoda* and ppm Pb in *Drosophila* ($r_s = -0.774$, $P < 0.001$, $N = 52$).

BOHÁČ J., RŮŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deteriorationis Regionis. Institute of Landscape Ecology CAS, České Budějovice, pp. 315-318.

Cd was biomagnified in both Drosophila and Steatoda. BCF for Drosophila were: 2.4 (a), 2.3 (b), 1.4 (c). BCF for Steatoda were: 57 (control), 5.9 (a), 6.9 (b), and 5.5 (c).

The efficiency of the spider's Cd consumption was $70 \% \pm 9.6$ (96 % confidence interval, N=46). There were no significant differences in the consumption efficiency between the different groups. There was an insignificant, correlation between ng Cd in Drosophila and ng Cd in the HNO_3 with which the vials were washed ($r_s = 0.164$, $P = 0.26$, $N = 49$) (Fig. 3).

The Cd content of prey remains was positively correlated with Cd in Drosophila ($r_s = 0.746$, $P << 0.0005$, $N = 51$).

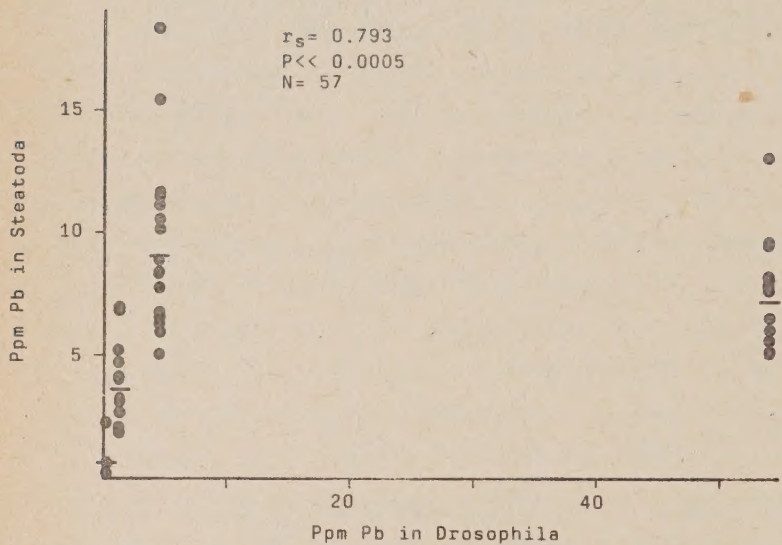


Fig. 1. Ppm (of dry-weight) Pb in Steatoda as a function of ppm Pb in Drosophila. The vertical bars are the geometrical means. r_s = Spearman's Rank Correlation Coefficient, N = sample size, P = probability under the nul hypothesis.

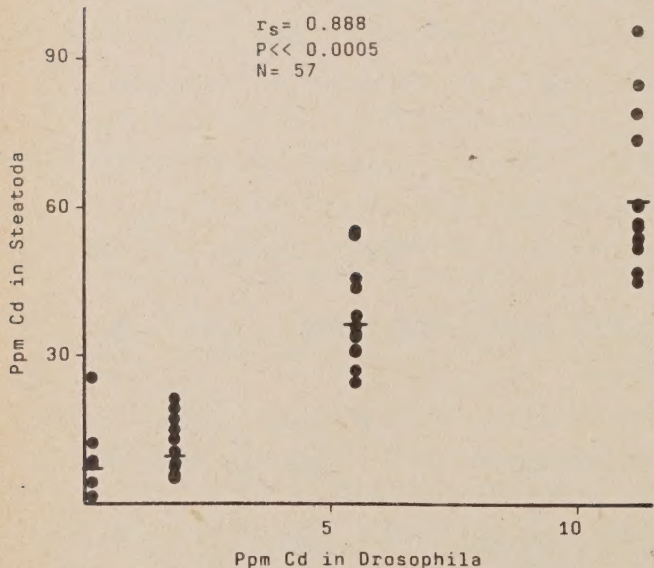


Fig. 2. Ppm (d. w.) Cd in Steatoda as a function of ppm Cd in Drosophila. The vertical bars are the geometrical means. r_s , N , and P as in Fig. 1.

DISCUSSION

Pb and Cd behave distinctly different, there being a dramatic decrease in Pb concentration from media to *Drosophila*, whereas Cd-concentrations increased markedly at each segment of the foodchain.

The present results strongly indicate that the Pb-BCF in *Steatoda* is dependant on the Pb concentration in *Drosophila*. This may be due to either a limited assimilation capacity for lead, or an increasing excretion efficiency with increasing environmental Pb-levels.

CLAUSEN (1984) noted high levels of Pb in *S. bipunctata* at polluted sites in Denmark (up to 100 ppm, geometric mean), and showed that about 25 % of the lead "in" *Araneus (Nuctenea) umbraticus* was deposited on the surface of the animals. Also, it was demonstrated that the spiders reflect the atmospheric Pb-pollution. The results thus indicate, that a major part of the lead found in free-living spiders originates from particles in the atmosphere. However, at lower environmental Pb-burdens input of Pb via prey may be of increasing importance.

Pb in *Steatoda* leveled off at lead concentration in *Drosophila* of about 5 ppm (dry weight). Cd, however, did not seem to have reached a plateau at cadmium contents in the flies above 10 ppm. - It is noteworthy that the highest lead concentration in *Drosophila*-media was about 42 times greater than that of Cd. - The levels of metals in the media are within the ranges found in freelifving organisms (HUGHES et al., 1980). Cd levels of say 50 ppm might thus be built up in spiders in polluted areas. Several bird species depend on spiders as food, especially during winter (JANSSON, BRÖMSSON, 1981; NORBERG, 1978), and 50 ppm Cd may certainly be a toxic concentration (SCHEUHAMMER, 1987; STOEWESAND et al., 1987); for example, the data of STOEWESAND et al. (1987) suggest a LC_{50} (14 weeks) of 0.2 - 0.3 ppm Cd in the food of *Coturnix coturnix japonica*.

The consumption efficiency for Cd of 70 % corresponds to the overall consumption efficiency of 67 %, indicating a 100 % assimilation efficiency of Cd, as there is a very low correlation

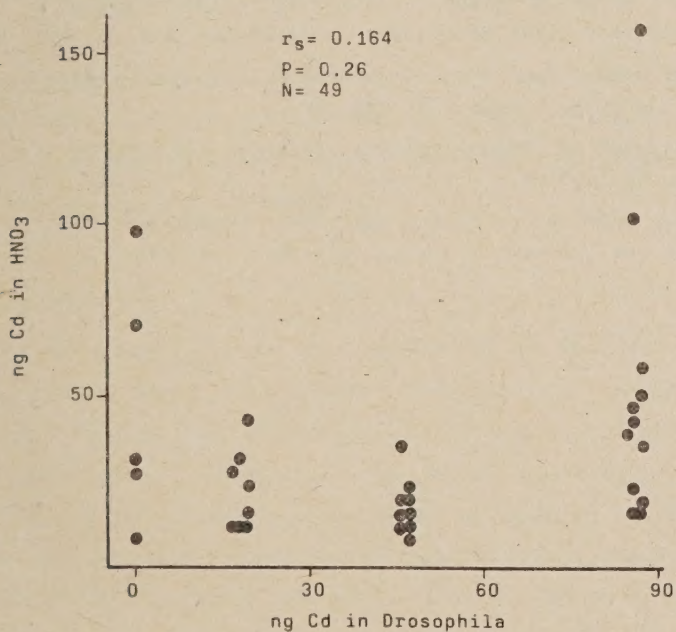


Fig. 3. Ng Cd in HNO₃ with which vials were washed as a function of ng Cd in *Drosophila*. r_s , N, and P as in Fig. 1.

between Cd in Drosophila and Cd in the nitric acid with which the vials were washed. The Cd in the HNO_3 compartment includes excreted Cd, Cd from the filter paper, and Cd from fragments of prey remains. Given the highly significant relation between Cd content of Drosophila and prey remains, the correlation between Cd in Drosophila and Cd excreted must be even less than 0.164.

C O N C L U S I O N S

Drosophila did not biomagnify Pb.

The Pb-BCF in Steatoda bipunctata is negatively correlated with the concentration in the prey, resulting in $\text{Pb-BCF} > 1$ at low doses, and < 1 at high doses.

Pb in Steatoda reached a level of 8 ppm (d. w.) at Pb concentrations in Drosophila of ca. 5 ppm.

A major part of the lead found in free-living spiders probably stems from atmospheric particles.

Cd was biomagnified in Drosophila as well as in Steatoda.

The Cd-consumption efficiency in the spiders was about 70 %. Cd-assimilation efficiency in Steatoda was close to 100 %, whereas the Cd-excretion was negligible.

Concentrations of Cd toxic (Chronic-) to spider-dependent birds may build up in spiders in polluted areas.

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Accumulation of heavy metals in the bodies of staphylinid beetles (Coleoptera, Staphylinidae)

J. BOHÁČ

Insects constitute a class of invertebrates that is extremely rich in species as well as individuals. Many insect species accumulate various chemical elements in their bodies.

Posing no major problems, collection of insects provides a large volume of material for chemical analysis. Owing to this, insects are employed to an ever-increasing extent as environmental pollution indicators (NEWMAN, SCHREIBER, 1984; POKARZHEVSKIJ, 1985; POKARZHEVSKIJ, BOHÁČ, ZHULIDOV, KUBIZŇÁKOVÁ, 1987).

Our insight into the accumulation of microelements by staphylinid beetles is so far insufficient. MOCHNACZKA-LAWACZ (1978) reports data of Fe, Sn, Cu, Mn and Mo contents of staphylinid beetle imagines collected at a fertilized meadow in Poland; her data, however, pertain to mixed samples from all the collected species of this family. However, the trophic specialization of species in a community can be diverse, and thus different microelement contents can be expected in different species. Previously we examined the microelement content of bodies of the large-size predatory staphylinid beetle Ocypus similis semialatus collected in Czechoslovakia over the time periods of the years 1900 - 1910 and 1946 - 1974. The material from the post-war period exhibited elevated concentrations of Sc, Sn and Cr (ZHULIDOV, BOHÁČ, POKARZHEVSKIJ, GUSEV, 1985). This suggested that other species also may be used for microelemental monitoring of the environment.

MATERIAL AND METHOD

The bodies of individuals of twenty-six staphylinid beetle species were subjected to analysis by AAS. The material was from four geographic regions: Bohemia Moravia (territory with an intensive agriculture and industry), the Rostov region of the USSR (agricultural landscape), the Caucasus mountain region and the Azores (protected territories) and northern Tadjikistan (agricultural landscape). The material had been collected over three time periods, viz 1900 - 1910, 1928 - 1948 and 1957 - 1974.

RESULTS

The microelement (except Hg) contents of the material from Czechoslovakia of 1957 - 1974 did not differ from those of 1900 - 1910 (Tab. 1). The Hg, Sn and Rb contents were highest in the 1928 - 1948 period, the Hg content was lowest in the 1957 - 1974 period.

The highest concentrations of mercury were observed in the material from the agricultural landscape in Bohemia (the north-west environment of Plzeň - Pilsen) and Moravia (the environment of Znojmo), whereas the lowest concentrations of this element were found in the material from forests in the vicinity of Prague and in the High Tatra. The elevated mercury levels in the material from agricultural landscape can be due to the seed for sowing having been treated with mercury containing agents.

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Tab. 1. Concentrations of chemical elements (mg/kg dry weight) in staphylinid beetles collected at various territories of Czechoslovakia over different periods of time.

Period	Hg	Sc	Zn	Co	Eu	Fe	Rb
1900-1910 (25 spec.)	13.4±3.3	0.04±0.02	728.6±429	0.21±0.03	0.03±0.01	734±872.6	3.15±1.23
1928-1948 (52 spec.)	17.1±18.1	0.02±0.01	1433.6±1476.8	0.2±0.09	0.03±0.09	428.1±313.8	4.9±3.6
1957-1974	7.1±8.3	0.06±0.06	508.9±415.3	0.25±0.19	0.03±0.01	623±519.7	3.18±1.30

Tab. 2. Concentrations of chemical elements (mg/kg dry weight) in staphylinid beetles collected at control sites (the Caucasus, the Azores) (1), in agricultural regions of Bohemia and Moravia (2) and in the industrial region of Prague and its environment (3) over the 1957 - 1974 period.

Region	Hg	Sc	Zn	Co	Eu	Fe	Rb
1 (38 spec.)	0.09±0.7	0.02±0.01	450±607	0.15±0.2	0.02±0.04	230±45	2.0±2.7
2 (58 spec.)	2.4±1.0	0.1±0.1	514±459	0.36±0.3	0.04±0.02	661±498	2.8±1.4
3 (61 spec.)	15.1±6.2	0.05±0.01	960±498	0.48±0.3	0.04±0.02	817±732	2.7±1.5

Tab. 3. Concentrations of chemical elements (mg/kg dry weight) in staphylinid beetles of different trophic groups collected in the Rostov region and in north-west Tadzhikistan over the 1957 - 1974 period.

Trophic group species	No. of specimens	Hg	Pb	Cd	Co	Mo
Zoophages						
Philonthus rectangulus	8	0.8±0.2	8.5±2.8	0.8±0.2	1.3±0.4	0.7±0.2
Philonthus concinnus	6	1.3±0.5	6.6±1.3	0.6±0.3	0.5±0.4	0.8±0.3
Philonthus quisquiliarius	8	2.6±0.5	8.5±2.2	0.3±0.1	2.4±1.0	0.5±0.1
Staphylinous dimidiaticornis	17	5.1±4.2	14.2±4.2	0.1±0.05	0.3±0.1	0.2±0.1
Ocypus similis semialatus	25	3.1±1.8	8.5±5.7	0.4±0.2	0.4±0.1	0.2±0.1
Phytophages						
Bledius fracticornis	3	1.8±0.4	1.9±0.3	0.1±0.05	3.8±0.5	0.7±0.4
Saprophages						
Oxytelus rugosus	8	1.3±0.7	4.8±2.2	0.2±0.1	4.5±1.8	-
Oxytelus sculpturatus	6	3.1±2.1	3.2±1.1	0.5±0.3	1.9±0.9	2.5±1.3
Platystethus cornutus	5	0.6±0.4	4.7±3.1	0.1±0.05	0.9±0.5	3.5±1.6
Mycetophages						
Oxyporus rufus	8	17.5±6.5	5.6±2.8	0.8±0.1	3.5±1.9	0.8±0.5

The data from industrial and agricultural regions compared with those from control (unpolluted) sites reveal a dependence of element contents on the degree of pollution for some chemical elements (Tab. 2). Against the control data, the mercury level in samples from

agricultural landscape was three times higher and from industrial landscape, nineteen times higher. On the other hand, no differences were observed for Eu, Rb and Co in samples from regions under different pollution stress.

The results of analysis confirmed the assumption that the degree of accumulation of microelements in the bodies of the beetles depends on their trophic patterns (Tab. 3). Elevated concentrations of lead were observed in some zoophagous species (6.6 - 8.5 mg/kg dry weight) and elevated concentrations of mercury, in the mycetophagous species of Oxyporus rufus (17.5 ± 8.5 mg/kg dry weight).

Elevated lead concentrations have also been found in other zoophages living in similar biotopes as staphylinids do, e. g. in carabids (ZOLOTAREVA, 1984). The elevated mercury levels in mycetophages can be due to the fact that some fungi are capable of accumulating this element (DIETL, 1987).

The study of the concentrations of chemical elements in the bodies of staphylinid beetles is at its beginning. Still, it is clear that insects of this family hold promise as indicators of the chemical composition of the environment and can be also employed for historical monitoring of microelement levels in the environment.

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Metal contents in the scots pine feeding moths *Dendrolimus pini* L. (Lep., Lasiocampidae), *Bupalus piniarius* L. and *Thera obeliscata* L. (Lep., Geometridae)

M. RANTATARO, J. LAINE, P. KOSKINEN, P. NUORTEVA

I N T R O D U C T I O N

It exists quite a lot of knowledge about the metal emissions from industry and other sources. It exists also considerable knowledge about the wet and dry depositions of the metals into the forest ecosystems, as well as about the fate of metals in the humus and mineral soil under pressure of the acid rain. On the contrary we know very little about the mode of distribution of the solubilized metals into the thousands of species of forest organisms. This knowledge is mainly restricted to those few species, which have been used as indicators for metal fallout. This situation is curious, because we need metal pollution knowledge before we know the fate and effects of the pollutants for the living organisms. Our laboratory has recently started a study about the fate of metals in the plants and animals in forests endangered by forest decline process (NUORTEVA, 1988). As a part of this series of studies PIHLAJAMAKI, VAANANEN, KOSKINEN et NUORTEVA (1988) studied the metal levels occurring in two sphingid moths, *Laotloe populi* on poplars and *Sphinx pinastri* on scots pine. They noted that adults of these two moths have rather similar metal contents although the metal contents of their food plants differ considerably.

We liked to have deepened knowledge about the specific variation in the metal contents of moths living as larvae on the same food plant. Therefore we studied the metal contents of three additional moths living on scots pine (*Pinus silvestris* L.) - the lasiocampid *Dendrolimus pini* and the geometrid *Bupalus piniarius* and *Thera obeliscata*.

M A T E R I A L S A N D M E T H O D

The moths were collected in the southern half of Finland by members of the Finnish Lepidopterological Society with light traps during the summer 1986 (Fig. 1). The trap consisted of a funnel-shaped illuminated white sheet through which the moths fall into a pot with poison. The collectors identified the requested species from their catches and sent the material for analyses. The total amount of observation points for the three species was 19 but there was no points where all three species were represented.

In the laboratory the samples were dried overnight in an oven at 105 °C. After drying the samples were weighed and digested in 5 ml concentrated nitric acid for 2 h at 50 °C and 16 - 18 h at 110 °C. After cooling 5 ml hydrogen peroxide was added and boiled 6 h at 110 °C. In the end the samples were filtered and diluted to 20 ml with distilled water. The samples so prepared were analysed for iron, zinc, manganese and copper with a Perkin Elmer 360 flame atomic spectrophotometer and for cadmium and aluminium with a Varian Spectral 30/40 graphite furnace atomic absorption spectrophotometer.

RESULTS

The results of analyses are given in Tab. 1. Some samples had so little mass that their results can't be regarded as fully reliable. So we have deleted these contents (those put in parenthesis) in further calculations.

There existed some variation, which was obviously connected to local pollution sources. For *D. pini* the highest iron, zinc, manganese and copper contents were found in Vehkalahti. There were also a fairly high aluminium contents in Vehkalahti and Imatra. For copper it existed no local differences.

T. obeliscata had fairly few differences between the observation points. However, the zinc and aluminium contents in the samples of Imatra and Lappeenranta were somewhat higher than in other samples. The copper content was very low in Lappeenranta, only 3 ppm, when the contents were elsewhere between 14 - 22 ppm.

B. piniarius showed high iron contents in Jurva (132 ppm) and in Siilinjärvi (104 ppm). The highest zinc content was in the sample of Hanko (241 ppm) whereas the lowest was in the nearby lying Tvarminne (187 ppm). The manganese content of *B. piniarius* showed all small variation (11 - 25 ppm) but, Sipoo was an exception with 65 ppm.

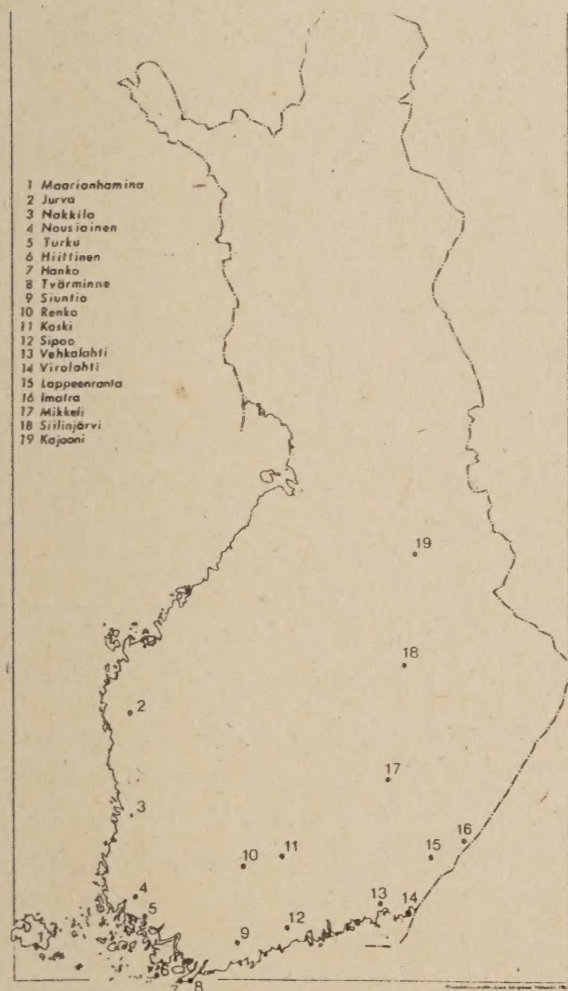


Fig. 1. Localities investigated.

Statistically there were significant differences between *D. pini* and *T. obeliscata* for both manganese and zinc contents ($p < 0.001$), with higher contents in the latter. *B. piniarius* showed statistically higher zinc contents than *D. pini*.

DISCUSSION

The metal contents of the adult moths showed considerable local variation but no clear areal trends. High peaks for some metals in some given localities reflect obviously local pollution sources. Moths are, however, not especially suitable indicator organisms, because our studies indicated that adult moths had lower metal contents than it existed in their food plant (except for Zn and Cu). The indicator suitability of moths is decreased by the fact that they are actively moving long distances and are collectable only with considerable labour. PIHLAJAMÄKI et al. (1988) noted that it is in the adult moths *Loathoe populi* and *Sphinx pinastri* lower contents of Al, Fe, Mn and Cd than in their respective food plants, but Zn and Cu occurred in elevated

Tab. 1. Heavy metal contents (mg/kg = ppm) in samples of Dendrolimus pini, Thera obeliscata and Bupalus piniarius at different observation points.

<u>D. pini</u>		Mean						
Observation point	N	weight, mg	Al	Fe	Zn	Mn	Cu	Cd
Nousiainen	2	2400	6	19	49	3	14	0.10
Turku	2	1800	14	12	79	4	18	-
Hanko	5	1000	7	52	71	9	17	-
Tvarminne	4	1800	7	22	89	4	15	-
Vehkalahti	5	1000	21	76	90	15	18	-
Virolahti	6	1800	12	47	74	9	15	-
Imatra	2	1100	29	48	68	7	17	0.15
Means		1600	14	39	74	7	16	0.04
Stand. dev.		480	9	23	14	4	2	0.06

<u>T. obeliscata</u>		Mean						
Observation point	N	weight, mg	Al	Fe	Zn	Mn	Cu	Cd
Turku (-)	3	43	-	-	205	61	15	-
Hiittinen	16	45	24	-	222	24	19	-
Siuntio (-)	2	51	19	-	268	49	-	-
Renko (-)	2	51	57	-	170	49	-	-
Koski Hl	9	61	47	-	213	28	14	-
Vehkalahti (-)	1	45	-	-	95	-	44	-
Virolahti	20	41	42	33	218	13	22	0.25
Lappeen-ranta	5	41	81	-	257	24	3	-
Imatra	50	35	54	25	255	22	20	0.30
Means		54	50	12	233	22	16	0.11
Stand. dev.		22	21	16	21	6	7	0.15

<u>B. piniarius</u>		Mean						
Observation point	N	weight, mg	Al	Fe	Zn	Mn	Cu	Cd
Maarian-hamina (-)	3	110	30	-	146	34	2	-
Jurva	18	84	42	132	194	11	9	-
Nakkila	8	79	63	53	220	15	24	-
Nousiainen (-)	3	93	42	-	192	34	12	-
Turku (-)	1	140	-	-	87	131	14	-
Hiittinen (-)	4	94	40	14	206	13	16	-
Hanko	8	99	40	23	241	14	13	-
Tvarminne	20	140	22	62	187	25	16	-
Sipoo	8	230	-	26	222	65	17	-
Mikkeli (-)	6	83	5	7	206	20	13	-
Siilinjärvi (-)	6	120	22	104	204	24	15	-
Kajaani (-)	1	54	-	160	130	317	10	-
Means		110	33	59	211	24	16	-
Stand. dev.		45	20	44	18	19	4	-

Tab. 2. Mean metal contents of *Deudrolimus pini*, *Sphinx pinastri* (PIHLAJAMAKI et al., 1988), *Thera obeliscata* and *Bupalus piniarius* in relation to adult weight and metal contents in the food plant.

Moths	Mean weight, mg	Al	Fe	Zn	Mn	Cu	Cd
<i>D. pini</i>	1600	14	39	74	7	16	0.04
<i>S. pinastri</i>	1900	16	86	172	4	16	0.09
<i>T. obeliscata</i>	54	50	12	233	22	16	0.11
<i>B. piniarius</i>	110	33	59	211	24	16	0.00
Host plant							
<i>Pinus silvestris</i>		200	95	53	180	2	0.22

contents. Our studies give good confirmation for their observation. PIHLAJAMAKI et al. (1988) assumed that the elevation of Zn in moths is connected to their ability to excrete effectively other metals. This comprehension is connected to the fact that Zn has this role in vertebrates (ELINDER, PISCATOR, 1978; MAILMAN, 1981; REDDY et al., 1987; YAMAMOTO et al., 1987). Quite different physiological mechanisms must, however, occur in the insects. It would be of interest to know, whether this secretory ability occurs already in the larvae or is connected to meconium excretion as observed in blowflies (NUORTEVA, 1982).

Our material consisted of moths of different size classes. If the sphingid moth *S. pinastri* from the study of PIHLAJAMAKI et al. (1988) is added to our material (Tab. 2), it is to see that the two really big species (*S. pinastri* and *D. pini*) have essentially smaller contents of metals in their bodies than have the more small-sized species (*T. obeliscata* and *B. piniarius*). This may be a size related effect, but may also depend on the preference of needles of different ages, which have different degrees of metal contamination (NUORTEVA et al., 1986; tables 3 and 4) or in different times of the season, because metal contents increase for most plants toward autumn (NUORTEVA, 1988). In accordance to SAALAS (1949) the most metal loaded *B. piniarius* devours old needles late in the summer whereas *D. pini* devours during its second year new needles in spring and early summer. This speaks for the comprehension that feeding habits would play a dominating role and the size a secondary role. - On the other hand it is to note that geometrid moths with small body mass connected with large surface collected most effectively radioactivity from the Chernobyl accident (MIKKOLA, ALBRECHT, 1986) and obviously also collect effectively metals too.

In addition to zinc the moths accumulated copper too. In small amounts copper is an essential metal securing the activities of several enzymes (FRIBERG et al., 1980). In model studies on the noctuid moth *Scotia segetum* it was shown that a moderate supply of copper elevated the copper contents in different developmental stages of the moth, caused protracted development of larvae but had no deleterious effects on pupae (ŠKROBÁK, WEISMAN, 1979; MAREŠOVÁ et al., 1977). ZELENAYOVA (1986) showed, however, that synthetic diet containing 3 µg CuCl₂/g caused characteristic disorders in the development and structure in the vitellogenic developmental stages of oocytes.

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Mussels as indicators of heavy metal pollution in the region of lake Balaton (Hungary)

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It is generally accepted that mussels are good indicator organisms both in lakes and rivers as well as in marine environment, due to their ability to accumulate and store various pollutants. They are used mainly for monitoring the presence of anorganic substances, like heavy metals (BUTLER et al., 1971; ROBERTS, 1976) but they are also suitable for detecting the occurrence of organic materials in the environment (GOLDBERG et al., 1978).

The use of mussels as biomonitors can be recommended for a number of reasons: (1) they are widespread in all the continents and climatic zones, (2) they are easily available and cheap, (3) the size of species is large enough to allow chemical measurement from a single animal or even from its organs separately, (4) they are sessile or comparatively slowly moving animals and so they stay all the time in their restricted environment, (5) they can be translocated from one place to another, (6) they are rather resistant to toxicants and unfavourable environmental conditions (change of t° , O_2 level, etc), (7) as filter-feeding animals they are in a very active, permanent contact with the environment both by feeding and respiration.

The behaviour of mussels is rather peculiar, namely, due to the presence of shells they are able to isolate themselves for shorter or longer period from the water (Fig. 1). This should be taken into consideration when using mussels as bioindicators, since their periodic activity may change under unfavourable conditions (SALÁNKI, 1965).

It is remarkable that some marine mussels do not show periodicity in their activity, e. g. *Pecten* and *Cardium* are not capable for longlasting tonic contraction ("catch") of their adductor muscles (SALÁNKI, 1966a, b) but freshwater species (*Anodonta*, *Unio*, *Dreissena*) all show alteration of active and rest periods.

In our studies we used *Anodonta* and *Unio* species, which are common in Lake Balaton. Five heavy metals, Cd, Cu, Hg, Pb and Zn were measured using atomic absorption spectrophotometric method (detailed description see in SALÁNKI et al., 1982) separately in different organs of the animal.

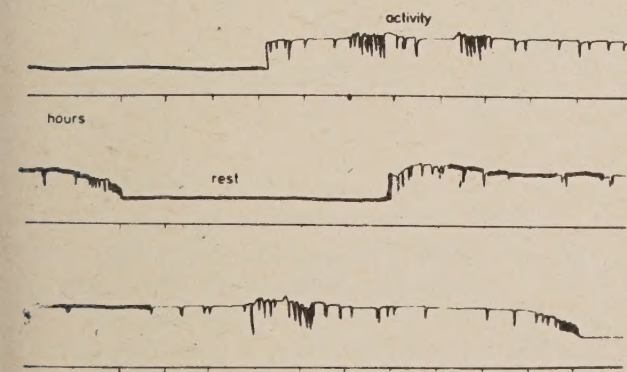


Fig. 1. Record of the activity of a mussel. During "activity" the shells are open and feeding, filtering take place. The pumping movements in this period are also recorded. During "rest" the shells are tightly closed. Both periods last for hours, and they are independent of any external fluctuation (BARNES, 1955; SALÁNKI, 1965; SALÁNKI et al., 1967).

Since our main interest was to clear up the heavy metal level of animals in Lake Balaton, we carried out a survey of the metal concentration in the gills, mantle, kidney, viscera and adductors of mussels collected at various locations of the Lake.

We found that the heavy metal contamination was highest in the gills and kidney, while in the muscles it was rather low (V.-BALOGH, SALÁNKI, 1984). Mussels collected in the region of the Keszthely Bay contained less heavy metal than mussels collected in the region of a boat-harbor, and these differences were rather significant referring to a moderate antropogenic pollution in the vicinity of boats treated with heavy metal containing paints (V.-BALOGH, 1988).

To monitor the heavy metal pollution in the region of Lake Balaton, we translocated mussels from the lake into three different regions of River Zala (Fig. 2). These locations were (a) before the town of Zalaegerszeg, (b) after the town, and (c) at the inlet into Lake Balaton. Mussels were collected in the Keszthely Bay. Samples were taken for heavy metal measurement with 2 weeks intervals for five months. We found rather great fluctuations in the concentration of all heavy metals in the gills and other organs, the results obtained in June and August are given at Tab. 1. The fact that not only increase but also decrease was detected during the investigated period suggests that (a) temporary pollutions of the water should occur and (b) mussels not only accumulate heavy metals but they also release them when the level of pollution decreases in the water.

To control the latter possibility we studied in laboratory conditions the uptake and depuration of heavy metals in different organs of mussels (SALÁNKI, V.-BALOGH, 1985). Differences were found in the uptake of various metals and organs in the very beginning of the exposure. As seen in Fig. 3. Hg is taken up by all the organs, while Cd was taken up only by the kidney at the first 24 hours. Further on a rather linear uptake takes place when animals are exposed to a moderate concentration of metals. Placing these animals into metal-free water, depuration starts immediately but the rate of release is different for various metals and different organs. As an example the uptake and release of Hg and Cd are shown in Fig. 4 for the gills of *Anodonta cygnea* L.

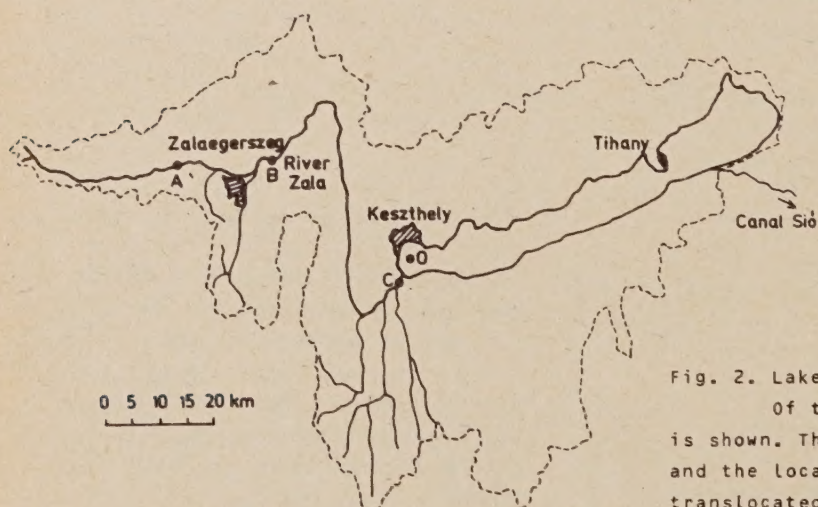


Fig. 2. Lake Balaton and its watershed.

Of the tributaries only river Zala is shown. The control sampling place (0) and the locations to where the mussels were translocated (A, B and C) are marked. Canal Sió is the single outflow of the lake.

Tab. 1. Concentration of heavy metals ($\mu\text{g/g}$ dry weight) in the gills of mussels at the place and time of collection, as well as at the place of translocation (for O, A, B and C see Fig. 2) after one and three months.

Location	Date	n	Hg	Cd	Fb	Cu	Zn
O	May 12	3	0.271 ± 0.063	4.38 ± 1.90	29.6 ± 2.27	10.6 ± 2.41	325 ± 5.33
A	Jun 8	3	0.119 ± 0.021	3.16 ± 1.05	29.8 ± 6.45	19.4 ± 3.68	344 ± 93.1
B		3	0.237 ± 0.054	12.1 ± 7.52	30.4 ± 4.01	150 ± 46.7	404 ± 107
C		3	3.67 ± 1.59	14.08 ± 0.01	20.6 ± 5.33	17.1 ± 1.96	439 ± 85.1
A	Aug 3	3	0.230 ± 0.130	2.00 ± 0.844	18.7 ± 7.56	20.2 ± 17.1	241 ± 92.4
B		3	0.400 ± 0.253	4.14 ± 1.39	20.8 ± 3.95	40.8 ± 15.6	357 ± 104
C		3	0.617 ± 0.174	3.26 ± 0.147	29.7 ± 1.00	10.1 ± 0.687	388 ± 91.4

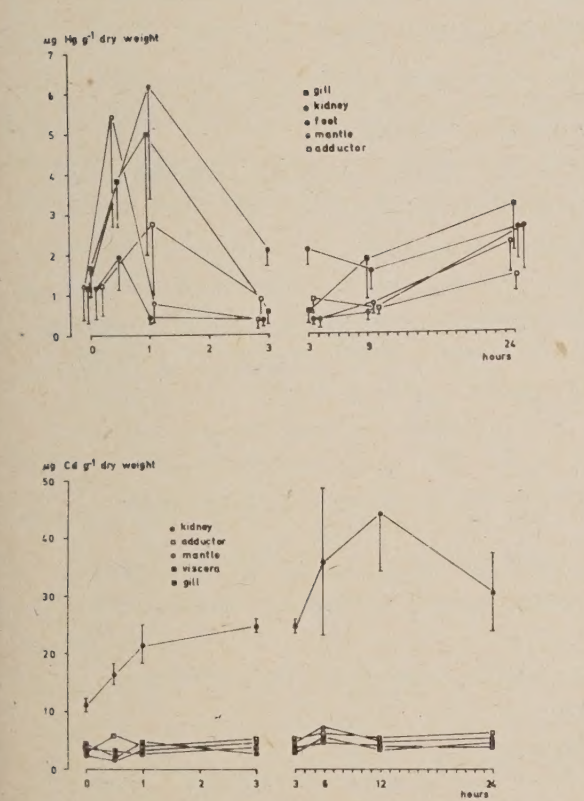


Fig. 3. Uptake of Hg (upper) and Cd (lower) into various organs of *Anodonta* in laboratory conditions. Note different scales for the first 3 hours and from 3 to 24 hours. Mercury and cadmium concentration of the water was 10 and 16 $\mu\text{g/L}$, respectively.

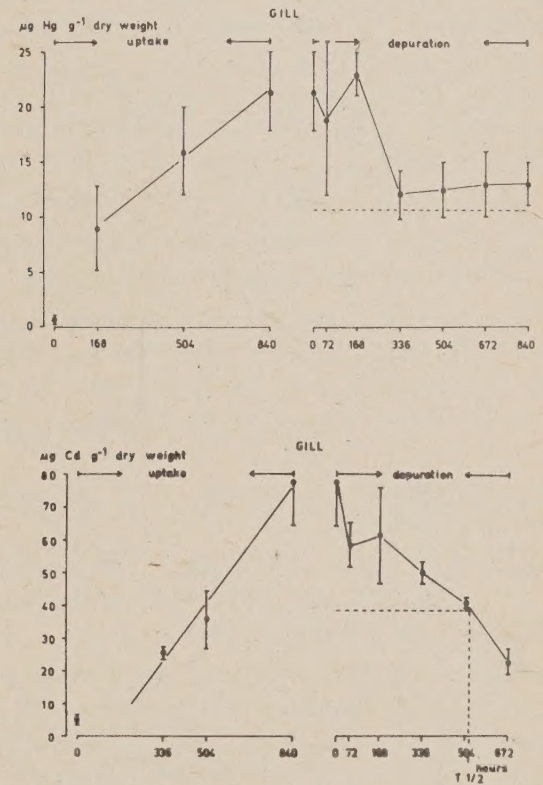


Fig. 4. *Anodonta* gill. Uptake and depuration of Hg (upper) and Cd (lower) in laboratory conditions. Dotted line during depuration marks 50 per cent level of release. Mercury and cadmium concentrations of the water during uptake are 12.0 ± 2.8 and 12.6 ± 1.3 $\mu\text{g/L}$, respectively.

It is clear from these experiments, that although heavy metals are washing out of the animal, nevertheless, the depuration is not complete within 4 - 5 weeks. This way, mussels are good objects not only for monitoring the general level of heavy metal pollution, but they are suitable organisms for biological monitoring of a temporary pollution wave, which is rather common not only in rivers, but may occur in each natural or man made surface water.

Using mussels as bioindicators we could establish a comparatively low metal pollution in Lake Balaton. A source of this pollution is River Zala through which time to time higher amount of heavy metals arrive into the lake. This is due certainly to the waste water of the town Zalaegerszeg, nevertheless, other point sources should be taken also in consideration. With the method of biomonitoring further survey may disclose these local polluting sources.

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Combined and separate effects of heavy metals on oxidative phosphorylation and mitochondrial respiration of the house cricket (*Acheta domestica* L.)

P. MIGULA

Zinc, lead and cadmium are metals of great importance in biological processes. Among them, only zinc is known to be required as an enzymatic cofactor, while lead and cadmium are non-essential and their importance derives from the fact that they are highly toxic (GEORGE, FRAZIER, 1982; MARONI, WATSON, 1985; BERGLIND, 1986). Their toxic action may exert through competition for binding sites on ligands in active centres of enzymes, mainly with sulphhydryl groups (BYCZKOWSKI, SORENSON, 1982). Usually the effects of a single metal at a time have been studied (MARTOJA et al., 1983; MEYER et al., 1986; MIGULA, in print). In a previous study it has been found that inhibitory effects of cadmium on metabolic functions of mitochondria of thoracic muscles and the fat body, were much more pronounced when metal was injected into the blood than ingested with food (MIGULA, 1986). In industrialized environment, however, organisms are exposed to a complex of various pollutants, including a mixture of heavy metals. The object of this study was to examine the pattern of interaction between Cd, Pb and Zn and its effect on mitochondrial functions in an insect (house cricket). The attention has also been paid on differences between insects contaminated by metals ingested with food, and insects directly injected with a solution containing heavy metals. Studies on respiratory metabolism in insects contaminated with heavy metals, as well as more detailed studies on enzymatic level (MIGULA, 1986), may be a useful method which allows for more approached prediction of metabolic fates of an organism, using the data of monitored metal concentrations in insects inhabiting variously contaminated environments.

MATERIAL AND METHODS

Adult male and female crickets of the same age, obtained from a stock colony, were maintained according to CLIFFORD et al. (1977) method. Two sets of experiments were carried out. In the first one insects were injected once with isotonic sodium chloride solution containing:

a: 100 μg Zn (1.53 μmol); b: 5 μg Pb (24 nmol); c: 2 μg Cd (18 nmol); d: 50 μg Zn + 2.5 μg Pb; e: 50 μg Zn + 1 μg Cd; f: 2.5 μg Pb + 1 μg Cd; g: 50 μg Zn + 2.5 μg Cd in the form of acetates/g body weight. Control crickets were injected with pure sodium chloride solution. Insects, in groups of 10, were maintained on a semisynthetic diet, in aluminium containers as described elsewhere (MIGULA et al., 1986). Seven days after injection they were used for respirometric analyses. In the second set of experiments crickets were kept for 7 days on diet containing a single metal or their combinations in the following concentrations g^{-1} dry weight of food: a: 1000 μg Zn (15.3 μmol); b: 50 μg Pb (240 nmol); c: 50 μg Cd (450 nmol); d: 500 μg Zn + 25 μg Pb; e: 500 μg Zn + 25 μg Cd; f: 25 μg Cd + 25 μg Pb; g: 500 μg Zn + 25 μg Pb + 25 μg Cd. Control insects were maintained on uncontaminated food. All insects were kept at 28°C and lighting schedule 12D:12L.

BOHÁČ J., RŮŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deteriorationis Regionis. Institute of Landscape Ecology CAS, České Budějovice, pp. 331-339.

Mitochondria were prepared from the thoracic muscles and the abdominal fat body. Thoraces cut into small pieces were suspended in a cold isolation medium containing Nagarse proteinase for 5 minutes. After filtration through cotton gauze mitochondrial fraction was isolated after differential centrifugation of the filtrate (CHAPPELL, HANSFORD, 1972). The fat body mitochondria were isolated according to the method of SUJAK (1984). Mitochondrial protein was determined by the method of BRADFORD (1976). The oxygen consumption was measured polarographically with a Clark-type oxygen electrode (ESTABROOK, 1967) at 25° C. The incubation medium (3 ml) for thoracic muscle mitochondria consisted of 10 mM Tris-HCl, pH 7.4, 25 mM potassium phosphate buffer, 154 mM KCl, 1 mM EDTA, 1 % albumin. The substrate was 10 mM pyruvate + 10 mM malate. ADP was added in a volume not exceeding 1 % of the total volume (WEEDA et al., 1980). The fat body mitochondria were added to 50 mM Tris-HCl, pH 7.4, 30 mM KH_2PO_4 , 15 mM KCl, 5 mM MgCl_2 , 1 mM EDTA, 0.3 % albumin. The substrate was 20 mM succinate. Both, ADP-stimulated (State 3) and ADP-limiting (State 4) oxygen consumption were monitored. Respiratory Control Ratio (R. C. R.), used as a measure of mitochondrial intactness, was calculated by the rate of State 3/State 4 respiration. The ratio of added ADP to the amount of oxygen used in State 3 expressed the oxidative phosphorylation. FCCP was used as the uncoupler. The concentrations of Cd, Pb and Zn in crickets were analysed by atomic absorption spectrophotometry.

R E S U L T S

Feeding with metal contaminated food or a direct injection of heavy metals into the blood stream has in almost all cases revealed in a significant increase in body burden of metals (Tab. 1, 2). The differences between sexes appeared to be insignificant. Concentration of metals were stated usually higher in insects fed with contaminated food, but, as it was indicated in other study (MIGULA et al., 1986), the superior parts of Cd, Pb and Zn were stored in the alimentary tract of crickets.

The effects of metals consumed with contaminated food on the respiration of thoracic muscle mitochondria, R. C. R. and oxidative phosphorylation are shown in Tab. 3. Cadmium and lead added singly to the diet inhibited significantly the State 3 respiration and slightly reduced both the R. C. R. and ADP: O ratio. The State 3 respiration was also reduced significantly in the thoracic muscle mitochondria isolated from insects kept on diet with the excess of two or three metals (Fig. 1, 2). Zinc in combination with lead or cadmium reduced toxicity of these metals. The ADP-limiting state of respiration remained unchanged (Tab. 3).

The effects of metals injected directly to the blood stream caused much more severe effects. With only one exception (zinc in male crickets), metals caused a significant reduction in the State 3 respiration. The reduction of oxygen uptake was of 16 to 47 % (Fig. 2). On the other hand, some stimulatory effects on the State 4 respiration were indicated (Tab. 4). Respiratory Control Ratio was visibly declined and reached in insects injected with Cd and Pb only a half of the value estimated for control crickets. Toxic effects of metals are also reflected in reduced ADP: O ratios. The reduction in respiratory rate in insects toxified with lead and cadmium was less pronounced when zinc was added with one of these metals. Such a "protective" effect of zinc was not indicated in crickets injected with a solution containing three metals (Fig. 1, 2).

Tab. 1. Concentration of zinc, cadmium and lead in control and contaminated crickets maintained on food with the excess of metals (nmol . g⁻¹ dry body weight ± S. D.). Asterisks denote statistically significant differences between contaminated and control crickets (+ P ≤ 0.05; ++ P ≤ 0.01; +++ P ≤ 0.001).

Sex	Group of crickets	Zinc	Cadmium	Lead
♂	Control	900.5 ± 111.3	27.4 ± 3.7	4.1 ± 1.6
	Zn	2420.0 ± 310.5 ⁺⁺⁺	31.8 ± 4.3	6.1 ± 1.5
	Pb	1150.0 ± 200.0 ⁺⁺⁺	23.8 ± 4.0	24.8 ± 1.9 ⁺⁺⁺
	Cd	960.0 ± 130.0 ⁺⁺⁺	64.1 ± 9.3 ⁺⁺⁺	3.2 ± 0.8
	Zn + Pb	1770.0 ± 218.0 ⁺⁺⁺	29.0 ± 3.5	11.4 ± 2.4 ⁺⁺⁺
	Zn + Cd	1910.0 ± 335.0 ⁺⁺⁺	47.3 ± 6.3 ⁺⁺⁺	5.1 ± 2.1
	Pb + Cd	1150.0 ± 160.0 ⁺⁺⁺	78.2 ± 8.9 ⁺⁺⁺	16.2 ± 3.5 ⁺⁺⁺
	Zn + Pb + Cd	2180.0 ± 338.0 ⁺⁺⁺	60.1 ± 7.1 ⁺⁺⁺	14.3 ± 1.1 ⁺⁺⁺
♀	Control	1033.0 ± 203.0	25.5 ± 2.3	3.8 ± 1.7
	Zn	2618.0 ± 143.0 ⁺⁺⁺	27.3 ± 3.8	4.7 ± 0.9
	Pb	980.0 ± 160.0	21.7 ± 3.9	21.5 ± 3.5 ⁺⁺⁺
	Cd	1206.0 ± 190.0	58.3 ± 7.5 ⁺⁺⁺	5.5 ± 1.0
	Zn + Pb	1811.0 ± 238.0 ⁺⁺⁺	24.3 ± 4.5	13.5 ± 1.9 ⁺⁺⁺
	Zn + Cd	1718.0 ± 240.0 ⁺⁺⁺	42.6 ± 6.3 ⁺⁺⁺	6.6 ± 1.1 ⁺⁺⁺
	Pb + Cd	908.0 ± 169.0	71.8 ± 8.1 ⁺⁺⁺	19.4 ± 2.8 ⁺⁺⁺
	Zn + Pb + Cd	1930.0 ± 310.0 ⁺⁺⁺	63.5 ± 8.1 ⁺⁺⁺	13.7 ± 1.4 ⁺⁺⁺

Tab. 2. Concentration of zinc, cadmium and lead in crickets injected with one of these metals or their combinations (nmol . g⁻¹ dry body weight ± S. D.). Asterisks denote statistically significant differences between contaminated and control crickets (+ P ≤ 0.05; ++ P ≤ 0.01; +++ P ≤ 0.001).

Sex	Group of crickets	Zinc	Cadmium	Lead
♂	Control	1070.0 ± 115.0	29.5 ± 3.3	5.6 ± 1.4
	Zn	1530.0 ± 300.0 ⁺⁺⁺	26.0 ± 5.0	7.2 ± 1.3
	Pb	1210.0 ± 211.0	27.1 ± 4.1	16.9 ± 3.6 ⁺⁺⁺
	Cd	1030.0 ± 160.0	43.5 ± 8.2 ⁺⁺⁺	4.8 ± 1.2
	Zn + Pb	1460.0 ± 212.0	24.1 ± 4.1	10.6 ± 3.3 ⁺⁺⁺
	Zn + Cd	1680.0 ± 246.0	35.0 ± 3.2 ⁺⁺⁺	6.6 ± 1.0
	Pb + Cd	990.0 ± 150.0	39.2 ± 6.1	13.4 ± 2.0 ⁺⁺⁺
	Zn + Pb + Cd	1820.0 ± 240.0 ⁺⁺⁺	36.6 ± 7.4 ⁺⁺⁺	10.8 ± 1.9 ⁺⁺⁺
♀	Control	950.0 ± 160.0	26.4 ± 2.8	5.0 ± 0.8
	Zn	1490.0 ± 159.0 ⁺⁺⁺	23.4 ± 4.2	4.3 ± 1.1
	Pb	1080.0 ± 201.0	26.2 ± 4.7	14.3 ± 1.9 ⁺⁺⁺
	Cd	1150.0 ± 166.0	38.7 ± 9.4 ⁺⁺⁺	4.0 ± 0.07
	Zn + Pb	1380.0 ± 190.0 ⁺⁺⁺	24.2 ± 5.1	11.8 ± 2.0 ⁺⁺⁺
	Zn + Cd	1470.0 ± 108.0 ⁺⁺⁺	32.5 ± 3.7 ⁺⁺	5.4 ± 0.6
	Pb + Cd	1060.0 ± 140.0	38.7 ± 6.5 ⁺⁺⁺	13.8 ± 2.6 ⁺⁺⁺
	Zn + Pb + Cd	1750.0 ± 208.0 ⁺⁺⁺	34.1 ± 7.2 ⁺	11.1 ± 1.9 ⁺⁺⁺

Tab. 3. The oxygen consumption (ng-atoms $O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ mitochondrial protein $\pm$ S. D.), oxidative phosphorylation (ADP : O) and the Respiratory Control Ratio of mitochondria isolated from the thoracic muscles of the house crickets maintained on heavy metal-contaminated food. Asterisks denote statistically significant differences between contaminated and control crickets ($^+ P \leq 0.05$; $^{++} P \leq 0.01$; $^{+++} P \leq 0.001$).

Sex	Group	N	State 3 (ADP-stimulated)	State 4 (ADP-limiting)	R.C.R.	ADP:O
♂	Control	10	250.4 $\pm$ 33.5	96.4 $\pm$ 11.8	2.6	2.5
	Zn	7	260.8 $\pm$ 21.7	99.2 $\pm$ 10.5	2.6	2.6
	Pb	8	230.2 $\pm$ 32.5	100.9 $\pm$ 12.5	2.3	2.4
	Cd	7	203.7 $\pm$ 19.2 ⁺⁺⁺	88.6 $\pm$ 12.5	2.3	2.5
	Zn + Pb	7	205.5 $\pm$ 11.9 ⁺⁺⁺	89.7 $\pm$ 13.3	2.3	2.4
	Zn + Cd	7	216.3 $\pm$ 21.2 ⁺⁺	87.2 $\pm$ 10.5	2.5	2.3
	Pb + Cd	7	190.1 $\pm$ 28.3 ⁺⁺⁺	90.5 $\pm$ 8.6	2.1	2.1
	Zn + Pb + Cd	7	179.1 $\pm$ 22.5 ⁺⁺⁺	90.4 $\pm$ 11.1	2.0	2.2
♀	Control	10	241.9 $\pm$ 18.4	89.2 $\pm$ 8.9	2.7	2.6
	Zn	7	237.5 $\pm$ 16.9	94.6 $\pm$ 11.5	2.5	2.5
	Pb	7	211.5 $\pm$ 25.1 ⁺⁺	99.3 $\pm$ 13.2	2.1	2.3
	Cd	8	187.5 $\pm$ 24.4 ⁺⁺⁺	85.2 $\pm$ 13.8	2.2	2.4
	Zn + Pb	7	223.7 $\pm$ 16.9	97.7 $\pm$ 10.8	2.4	2.2
	Zn + Cd	7	215.9 $\pm$ 18.7 ⁺⁺	87.1 $\pm$ 11.8	2.6	2.2
	Pb + Cd	6	203.3 $\pm$ 19.5 ⁺⁺⁺	96.8 $\pm$ 15.8	2.1	2.3
	Zn + Pb + Cd	7	185.1 $\pm$ 33.2 ⁺⁺⁺	93.5 $\pm$ 13.5	2.1	2.1

Tab. 4. The oxygen consumption (ng-atoms $O_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ mitochondrial protein $\pm$ S. D.), oxidative phosphorylation (ADP : O) and the Respiratory Control Ratio of mitochondria isolated from the thoracic muscles of the house crickets injected with one of the metals or their combinations. Asterisks denote statistically significant differences between contaminated and control crickets ($^+ P \leq 0.05$; $^{++} P \leq 0.01$; $^{+++} P \leq 0.001$).

Sex	Group	N	State 3 (ADP-stimulated)	State 4 (ADP-limiting)	R.C.R.	ADP:O
♂	Control	10	220.8 $\pm$ 30.0	90.5 $\pm$ 15.1	2.4	2.6
	Zn	9	210.5 $\pm$ 18.5	101.2 $\pm$ 18.9 ⁺	2.1	2.4
	Pb	9	169.1 $\pm$ 15.9 ⁺⁺⁺	106.5 $\pm$ 13.7	1.6	2.1
	Cd	9	177.3 $\pm$ 19.9 ⁺⁺⁺	104.1 $\pm$ 21.5 ⁺	1.7	1.9
	Zn + Pb	8	158.3 $\pm$ 30.6 ⁺⁺⁺	107.0 $\pm$ 15.9	1.5	2.3
	Zn + Cd	8	184.3 $\pm$ 20.8 ⁺⁺	103.0 $\pm$ 24.8	1.8	2.0
	Pb + Cd	8	155.2 $\pm$ 18.7 ⁺⁺⁺	133.1 $\pm$ 22.6 ⁺⁺	1.2	1.8
	Zn + Pb + Cd	8	151.2 $\pm$ 21.3 ⁺⁺⁺	108.2 $\pm$ 21.5	1.4	1.9
♀	Control	10	230.3 $\pm$ 21.1	84.1 $\pm$ 13.2	2.7	2.6
	Zn	8	193.2 $\pm$ 15.2 ⁺⁺⁺	102.3 $\pm$ 15.5 ⁺⁺	1.8	2.5
	Pb	9	170.1 $\pm$ 16.9 ⁺⁺⁺	101.5 $\pm$ 16.9 ⁺⁺	1.9	2.0
	Cd	9	177.3 $\pm$ 19.9 ⁺⁺⁺	104.1 $\pm$ 21.5 ⁺	1.7	1.9
	Zn + Pb	7	162.2 $\pm$ 21.2 ⁺⁺⁺	89.3 $\pm$ 18.8	1.7	2.4
	Pb + Cd	8	129.0 $\pm$ 31.2 ⁺⁺⁺	102.6 $\pm$ 11.6 ⁺⁺⁺	1.3	2.1
	Zn + Pb + Cd	7	144.3 $\pm$ 17.9 ⁺⁺⁺	105.4 $\pm$ 14.7 ⁺⁺⁺	1.4	2.1

Cadmium appeared to be the most toxic for the metabolic functions of the fat body mitochondria (Tab. 5). The oxygen uptake by mitochondria isolated from intoxicated crickets was reduced in the State 3 to 75 - 90 % of the control level (Fig. 1, 2). The R.C.R. for the fat body mitochondria was less affected than in sarcosomes, but ADP : O ratio dropped visibly, reaching hardly a half of the control value in insects injected with three metals.

A correlation between metal body burden and State 3 respiration was stated to be significant in almost all cases for cadmium, as well as for lead and female fat body mitochondria (Tab. 6).

D I S C C U S I O N

Lead is accumulated passively and actively by mitochondria and often is competitive to Ca^{+2} . Cadmium cations also bind to the mitochondrial membranes, similar as those of zinc (BRIERLEY, 1976). The order of effectiveness with respect to concentration was stated as follows: $\text{Pb}^{+2} \gg \gg \text{Cd}^{+2} \gg \text{Zn}^{+2}$. Inhibitory effects of metals may be reversed by use of chelating agents (KLEINER, 1974; DIAMOND, KENCH, 1974), but in the present study addition of EDTA did not change oxygen uptake by poisoned mitochondria (Tab. 3 - 5).

Metals introduced into the organism as ionizable salts are usually non-competitive inhibitors of oxido-reducing and transporting enzymes, especially those rich in free-SH groups (BYCZKOWSKI, SORENSON, 1984). It is known from the studies on mammals that the respiratory chain can be inhibited by heavy metals at different sites (DIAMOND, KENCH, 1974; KLEINER, 1974). These mechanisms are also responsible for quantitative changes in the metabolism of both fat body and thoracic muscle mitochondria of crickets toxified with binary combinations of metals. Suppressed efficiency of oxidative phosphorylation in insect mitochondria toxified with cadmium has also been stated (MIGULA, in print).

In insects body burden with metals is related to the balance between its uptake and excretion. Despite of proportionally lower amounts of metals which entered the body of crickets intoxicated by injection method (rather unnatural), the rate of their excretion was slower, thus higher concentrations remained in their body (Tab. 1, 2). It is obvious that physiological role of metals depends on their eventual compartmentation in the body. POLEK et WEISMANN (1984) stated, for example, that more than 95 % of total retained cadmium is stored in the digestive tract of larva. HOPKIN (1986) studying ecophysiological strategies of insects for surviving heavy metal pollution, has clearly shown that the ultrastructure of the midgut epithelial cells is responsible for absorption of potentially toxic metals from the food in the lumen to the blood. Previous experiments carried out on crickets toxified with cadmium (MIGULA, in print), and the present study have shown that a single injection of metal into the blood stream caused more severe changes in metabolic functions of mitochondria, than even higher amounts of metals consumed with contaminated food. Crickets exposed to both Cd and Pb showed more pronounced decrease in activity of mitochondrial metabolism than it was observed in insects exposed to one of these metals in equivalent concentrations.

Prevention of cadmium by zinc was visibly indicated in studies on mammals (MERALI et al., 1976; NAKAMURA et al., 1979). It is worth to note that in the presence of Zn both Cd and Pb were cumulated in proportionally lower rates (Tab. 1, 2). The inhibitory effects of Pb and Cd on the State 3 respiration of mitochondria were also slightly prevented in the presence of Zn.

Tab. 5. The oxygen consumption ($\text{ng-atoms O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ mitochondrial protein $\pm$ S. D.), oxidative phosphorylation (ADP:O) and the Respiratory Control Ratio of mitochondria isolated from the fat body of the house crickets injected with one of the metals or their combinations. Asterisks denote statistically significant differences between contaminated and control crickets ($^+ P \leq 0.05$; $^{++} P \leq 0.01$; $^{+++} P \leq 0.001$).

Sex	Group	N	State 3 (ADP - stimulated)	State 4 (ADP - Limiting)	R.C.R. ADP:O	
σ^7	Control	10	45.1 ± 7.2	16.3 ± 2.7	2.8	2.4
	Zn	9	47.2 ± 3.8	16.8 ± 4.0	2.8	2.5
	Pb	9	$36.3 \pm 8.3^+$	12.5 ± 3.3	3.4	2.0
	Cd	7	$30.8 \pm 3.9^{+++}$	14.0 ± 2.8	2.2	1.4
	Zn + Pb	8	$37.8 \pm 6.7^+$	14.4 ± 2.9	2.6	2.0
	Zn + Cd	8	$36.9 \pm 5.8^{++}$	15.5 ± 2.7	3.1	2.3
	Pb + Cd	7	$31.1 \pm 6.9^{+++}$	14.0 ± 1.9	2.2	1.8
	Zn + Pb + Cd	7	$33.8 \pm 6.2^{+++}$	14.7 ± 1.9	3.3	1.4
ϕ	Control	10	40.4 ± 4.4	13.2 ± 3.1	3.1	2.6
	Zn	7	$47.7 \pm 8.3^+$	12.2 ± 1.9	3.9	2.4
	Pb	8	$31.0 \pm 4.9^{+++}$	10.6 ± 2.1	2.9	2.1
	Cd	8	$31.7 \pm 6.2^{+++}$	13.2 ± 2.8	2.4	1.2
	Zn + Pb	7	$36.3 \pm 1.7^+$	11.5 ± 3.0	3.1	2.0
	Zn + Cd	7	$34.8 \pm 6.9^+$	11.9 ± 2.4	2.9	2.4
	Pb + Cd	7	$31.8 \pm 4.9^{+++}$	9.8 ± 1.9	3.2	1.9
	Zn + Pb + Cd	7	$35.0 \pm 5.1^{++}$	10.2 ± 2.2	3.4	1.4

Tab. 6. Relationship between ADP-stimulated mitochondrial respiration ($Y = \text{ng atoms O}_2 \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ mitochondrial protein) and a metal body burden ($X = \text{metal concentration in nmol} \cdot \text{g}^{-1}$ dry weight).

r - correlation coefficient; I - insects injected; F - insects fed with the excess of metals.

Sex	Metal	Tissue	I/F	$Y = aX - b$	r	Significance
σ^7	Zn	Thoracic muscle	I	$300.96 - 7.68 E^{-2}X$	- 0.706	$P \geq 0.05$
			F	$247.0 - 1.34 E^{-2}X$	- 0.232	$P \geq 0.05$
		Fat body	I	$61.04 - 1.38 E^{-2}X$	- 0.67	$P \geq 0.05$
	Pb	Thoracic muscle	I	$222.28 - 4.478X$	- 0.648	$P \geq 0.05$
			F	$225.08 - 0.990X$	- 0.952	$P \leq 0.01$
		Fat body	I	$45.2 - 0.690X$	- 0.690	$P \geq 0.05$
	Cd	Thoracic muscle	I	$307.32 - 3.524X$	- 0.654	$P \geq 0.05$
			F	$277.26 - 1.251X$	- 0.869	$P \leq 0.01$
		Fat body	I	$74.75 - 1.066X$	- 0.67	$P \geq 0.05$
ϕ	Zn	Thoracic muscle	I	$322.46 - 0.102X$	- 0.886	$P \leq 0.05$
			F	$230.41 - 5.25 E^{-3}X$	- 0.132	$P \geq 0.05$
		Fat body	I	$45.06 - 4.38 E^{-3}X$	- 0.233	$P \geq 0.05$
	Pb	Thoracic muscle	I	$265.07 - 8.739$	- 0.838	$P \geq 0.05$
			F	$240.24 - 1.886X$	- 0.606	$P \leq 0.01$
		Fat body	I	$45.74 - 0.968X$	- 0.952	$P \leq 0.01$
	Cd	Thoracic muscle	I	$369.43 - 5.874X$	- 0.770	$P \leq 0.05$
			F	$263.08 - 1.076X$	- 0.849	$P \leq 0.01$
		Fat body	I	$58.03 - 0.683X$	- 0.986	$P \leq 0.01$

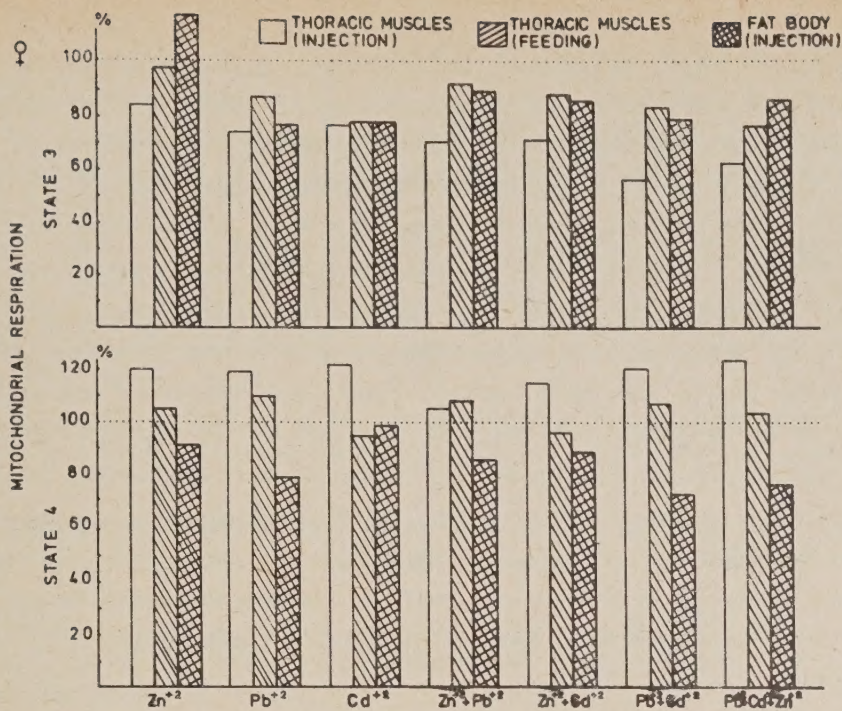


Fig. 1. Effects of metals on mitochondrial oxidation rates of pyruvate + malate (thoracic muscles) and succinate (fat body) in male crickets exposed to heavy metals in relation to control.

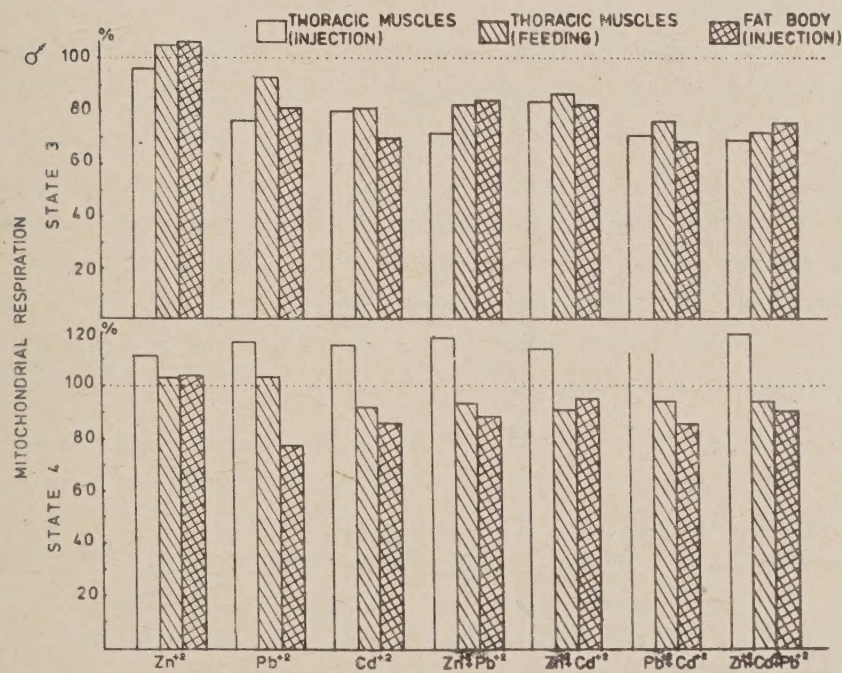


Fig. 2. Effects of metals on mitochondrial oxidation rates of pyruvate + malate (thoracic muscles) and succinate (fat body) in female crickets exposed to heavy metals in relation to control.

Nevertheless, preventive action of zinc has not been stated in insects exposed to tertiary combination of these metals (Fig. 1, 2). The present results show that reduction of the State 3 respiration in the fat body mitochondria by cadmium, as well as binary or tertiary combinations of used metals, was much more pronounced in males. General function of the body in adult male crickets was the storage of reserve materials, while cells of the fat body in a female of the same age are engaged mainly in the synthesis of materials used for egg production (MARTOJA et al., 1983). These physiologically different features may explain why female body cells are less sensitive to the metals than cells of the male (MARTOJA et al., 1983; MIGULA, in print.). On the other hand, thoracic muscle mitochondria of females appeared to be more sensitive to the injected metals (Tab. 4). It is known from the study of CYMBOROWSKI et DUTKOWSKI (1970) that spontaneous motoric activity is far greater in females. Thus it is possible that, in a similar way as described for skeletal muscles of mammals poisoned with heavy metals, toxic effects of metals are much more pronounced in muscles of more active females (JETHON et al., 1988). Studies of MARTOJA et al. (1983) on toxicity of cadmium in *Locusta* were shown, however, that after maturation females appeared to be less sensitive to cadmium and their mortality rate was lower than in males.

C O N C L U S I O N S

1. The overall effect of potentially toxic metals, injected or consumed with food, on coupled mitochondria isolated from thoracic muscles and the fat body of adult crickets is reflected by the inhibition of the ADP-stimulating respiration, decreased Respiratory Control Ratio and reduction of ADP : O ratio.
2. Among metals administered separately, cadmium appeared to be the most toxic, especially after its immediate injection into the blood stream.
3. Enhancement of these effects followed the administration of metals in binary or tertiary combinations.
4. Zinc reduced toxic effects of both cadmium and lead, with the exception of the simultaneous excess of three metals.
5. The fat body mitochondria in male appeared to be more sensitive to metal toxicity, than these of the female crickets. Reversely, toxicity of metals was much more pronounced in the mitochondria isolated from thoracic muscles of the female.

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Heavy metal contents and adenylate energy charge in insects from industrialized region as indices of environmental stress

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I N T R O D U C T I O N

Environmental contamination constitutes nowadays more severe problem than any time before, leading in many cases to a pollution disaster. Thus, investigations are needed, which allow not only to estimate concentrations of pollutants, but also their impact on living organisms. This problem is of a special importance in Upper Silesia, which is the most industrialized region in Poland. Plants expel large quantities of metal-containing dust. These metals become incorporated in animal and plant tissues affecting them both directly and indirectly (MIGULA, 1985). More attention has been paid recently on insects as indices of ecosystem pollution. Effects of air pollutants on insect populations had been reviewed by ALSTAD et al. (1982). Ecotoxicology of heavy metals in insects inhabiting grasslands has been studied recently by HUNTER et al. (1987a, b), while in forest insects by HELIOVAARA (1986), HELIOVAARA et VAISANEN (1986), STRAALEN et WENSEM (1986), HELIOVAARA et al. (1987), STARÝ et KUBIZŇÁKOVÁ (1987). In most of these studies, however, insect abundance, density gradient or contamination with heavy metals were analyzed in relation to a single pollution source. This study is an attempt to a potential use of honey bees and some other insect species, as well as honey and pollen, as biomonitors of environmental contamination. Honey bees have been reported to concentrate elevated levels of heavy metals (STOEWSAND et al., 1987). The foraging area of honey bees extends to about four kilometers, and within this range it is easy to indicate main sources of pollution (JONES, 1987). The analysis of pollen, collected by honey bees, provides further information on contamination of visited plants (TONG et al., 1975; FREE et al., 1983; JONES, 1987). Concentrations of heavy metals in honey bees, pollen and honey measured in samples taken in monthly intervals during consecutive seasons from variously contaminated sites might be next compared with available data on dust fall-out and meteorological indices. Organisms residuing in contaminated areas are subjected to a complex mixture of various pollutants and consequently have developed a number of biochemical mechanisms for survival (GEORGE, FRAZIER, 1982). Metabolic response of an organism to environmental stressors may be reflected by the level of the Adenylate Energy Charge (AEC) and a total pool of adenine nucleotides (ATKINSON, 1977). These parameters have also been proposed as general indices of metabolic homeostasis in insects exposed to various levels of environmental stressors (GIESY et al., 1983; HAYA, WAIWOOD, 1983).

M A T E R I A L A N D M E T H O D S

Six sites of the Upper Silesian Industrialized Region (Southern Poland) were chosen, as depicted in Fig. 1. Ruda Śląska, Chorzów and Jaworzno are strongly polluted from local coal-mines, steel works power plants and many other factories. Tworóg and Tworóg-Koty are mainly exposed

BOHÁČ J., RŮŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deteriorationis Regionis. Institute of Landscape Ecology CAS, České Budějovice, pp. 340-349.

to emissions expelled from various sources, located South and South-East of these sites. Among selected localities, Pszczyna is less polluted, as the main pollution comes from a moderate dust fall-out and road traffic.

Honey bees were collected in monthly intervals from each site in 1986 and 1987. Internal organs were dissected, weighed, dried and mineralized. The digests were analysed for Zn, Cu, Cd and Pb by atomic absorption spectrophotometry. Honey bees used for adenylate determinations were weighed and freeze-clamped at -196°C in liquid nitrogen. Tissues were ground to a fine powder in liquid nitrogen (HESS, BRAND, 1974). Similar procedure was applied for other species: *Philaenus spumarius* L. and *Aphis fabae* (Homoptera), representing saap feeders and *Chorthippus* spp. (Orthoptera) as representatives of grazing herbivorous insects. ATP and ADP/AMP concentrations were determined by the method of BUCHER (1947) and JAWOREK et al. (1974) respectively. The Adenylate Energy Charge (AEC) was calculated as follows:

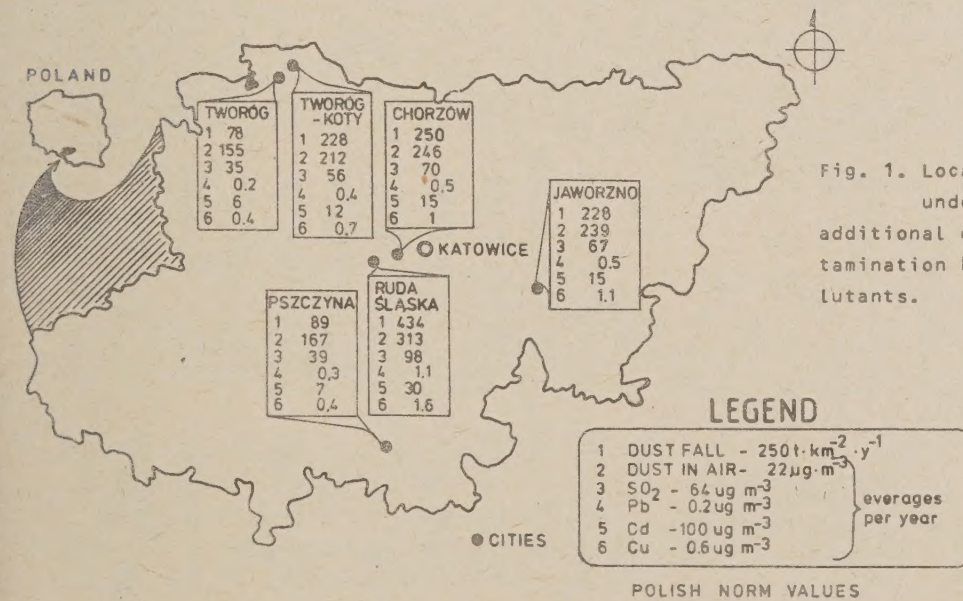
$$\text{AEC} = \frac{\text{ATP} + 1/2 \text{ ADP}}{\text{ATP} + \text{ADP} + \text{AMP}}$$

where ATP, ADP and AMP are adenine triphosphate, diphosphate and monophosphate respectively (ATKINSON, 1977).

RESULTS

Metal accumulation in honeybees

On the basis of data obtained for 1986 and 1987 from the Southern Poland web monitoring system, sites selected for our study may be ranked in decreasing order of environmental contamination as follows: Ruda Sleska $\geq$ Chorzów $\geq$ Tworóg-Koty $\geq$ Jaworzno $\geq$ Pszczyna $\geq$ Tworóg (Fig. 2). Only a partial correlation was stated between contents of heavy metals in the air and body burden with copper or lead in honey bees (Fig. 2).



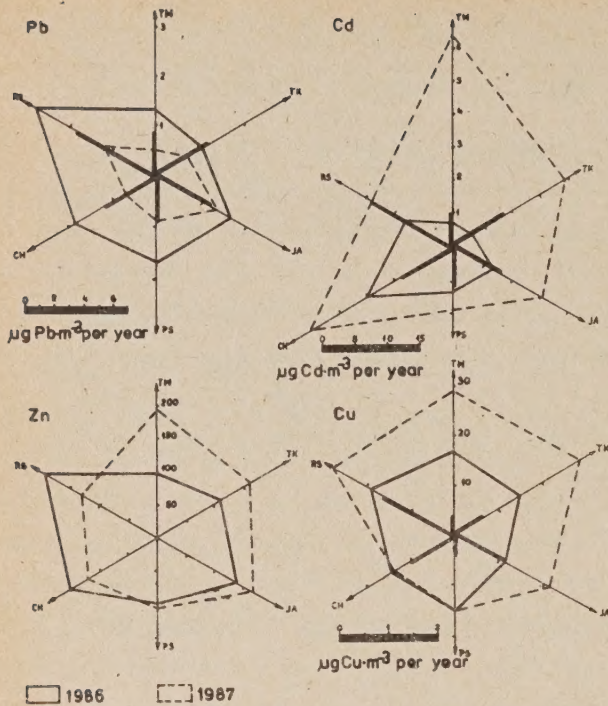


Fig. 2. Contents of four metals in the body of honey bees ($\mu\text{g/g}$ dry body weight) collected from various sites of Upper Silesian Industrial Region compared with average annual concentration of metals in the air dust. Abbreviations: RS - Ruda Śląska; TW - Tworóg; TK - Tworóg-Koty; JA - Jaworzno; PS - Pszczyna and CH - Chorzów.

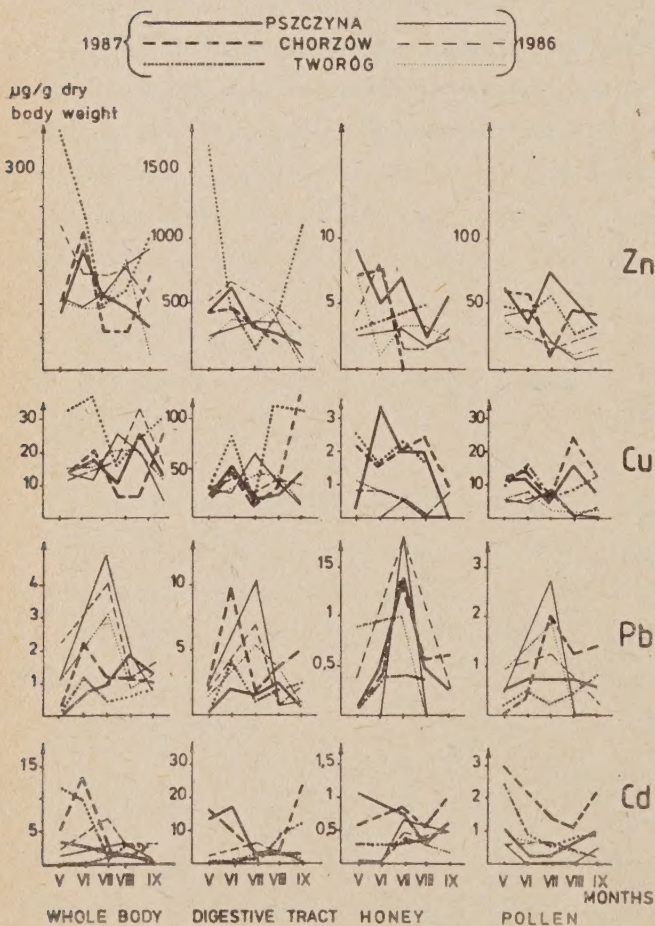


Fig. 3. Changes of four metal contents in the body of honey bees, their digestive tracts, pollen and honey from selected sites during two consecutive seasons.

The extent to which various organs take up metals differs significantly. The highest concentrations was in the gut of the honey bees, while the lowest in thoracic muscles. Body burdens with metals varied also within consecutive months of the season (Fig. 3). Thus annual averages fit better with the data for environmental contamination (Fig. 4). Annual averages of zinc concentration in honey bees, (especially in the gut), fit well with the fall-out of dust in selected sites, (Fig. 1, 2), except of high Zn concentration in Tworóg (1987). Such relationship was not stated for Zn content in honey and pollen. (Fig. 4). Maximal concentrations of Cu in honey bees were stated for Ruda Slaska, while the lowest for Tworóg-Koty (1986) and Pszczyna (1987), but there was no correlation between the Cu contents in insects and honey or pollen (Fig. 4). The amount of Pb cumulated in honey bees was generally lower in 1987 than in 1986, but the highest concentrations of this metal were also stated in insects collected from mostly contaminated sites (Fig. 4). Opposite to Pb, body burden with Cd was higher in 1987 and exceeded 9 times the concentration stated in insects collected from Tworóg in 1986. Generally higher values were also stated in samples of pollen and honey from various sites. A positive correlation was found between overall concentration of cadmium in studied areas and Cd content in the digestive tract, but only for 1987.

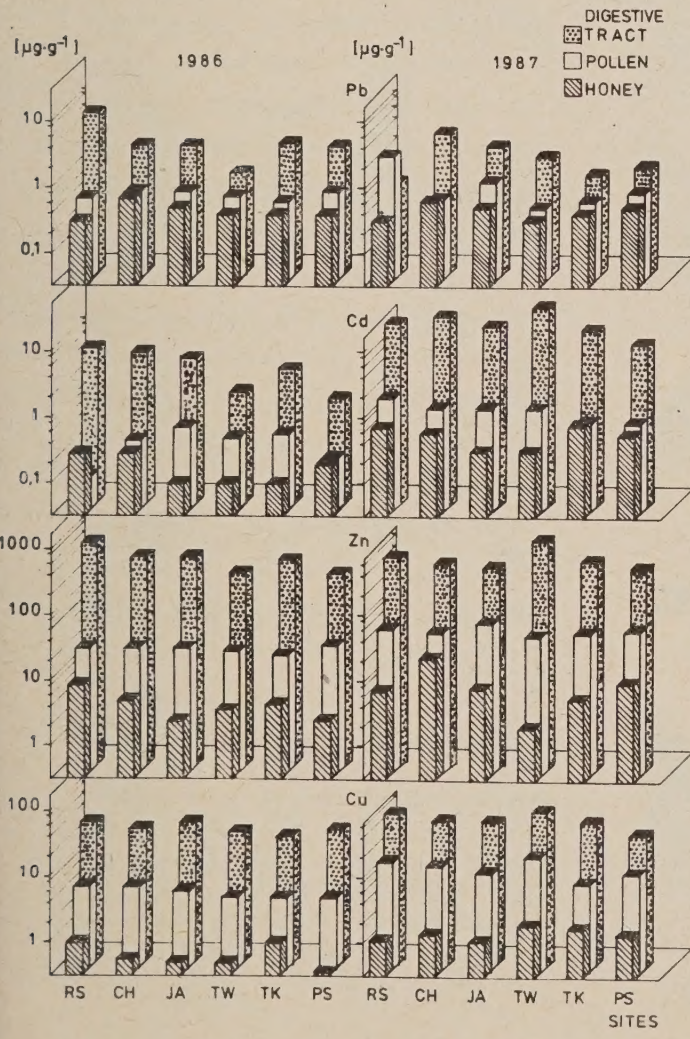


Fig. 4. Contents of four metals in the digestive tract of the honey bee, pollen and honey collected at various sites; annual averages in $\mu\text{g}\cdot\text{g}^{-1}$ dry weight for two consecutive years.

Measurements of adenylate pool in honey bees showed a distinct variability within and between the seasons, as it is depicted for selected sites in Fig. 5 and 6. The highest level of adenylates and the AEC was stated in insects from Pszczyna, while the lowest in insects from Chorzów (Fig. 7). Decreased adenylate concentrations in honey bees from Tworóg in August 1987 followed their elevated body burdens with heavy metals. Both, total concentration of nucleotides and the AEC, are not corresponding to the variations in dust fall-out and other factors characterizing the contamination level of studied areas. This means that many other environmental factors might be also responsible for observed pattern of nucleotide changes in honey bees.

Concentration of adenylate nucleotides and the AEC measured in other species of insects indicated generally higher levels for herbivorous chewers represented by *Chorthippus brunneus* and *Ch. biguttatus*, in comparison with the herbivorous sap feeders (*Philaenus spumarius* and *Aphis fabae*) and a mellitophage (Fig. 6).

DISCUSSION

Upper-Silesian Industrial District has remained for over a hundred years under interrupted pressure of industrial emissions, which comes mainly from the local sources (BARTKIEWICZ, RADECKA-MIKULICZ, 1972). Small distances between emitters, obsolete technologies and unefficiently working filters allow to classify this region to the most polluted areas in Europe. Thus, it might be expected that in such conditions even a rough bioindication of the pollution level in honey bees and other insects could more precisely reflect contamination levels within their foraging areas. On the other hand, such estimates could not sufficiently assess the effects of pollutants on these important

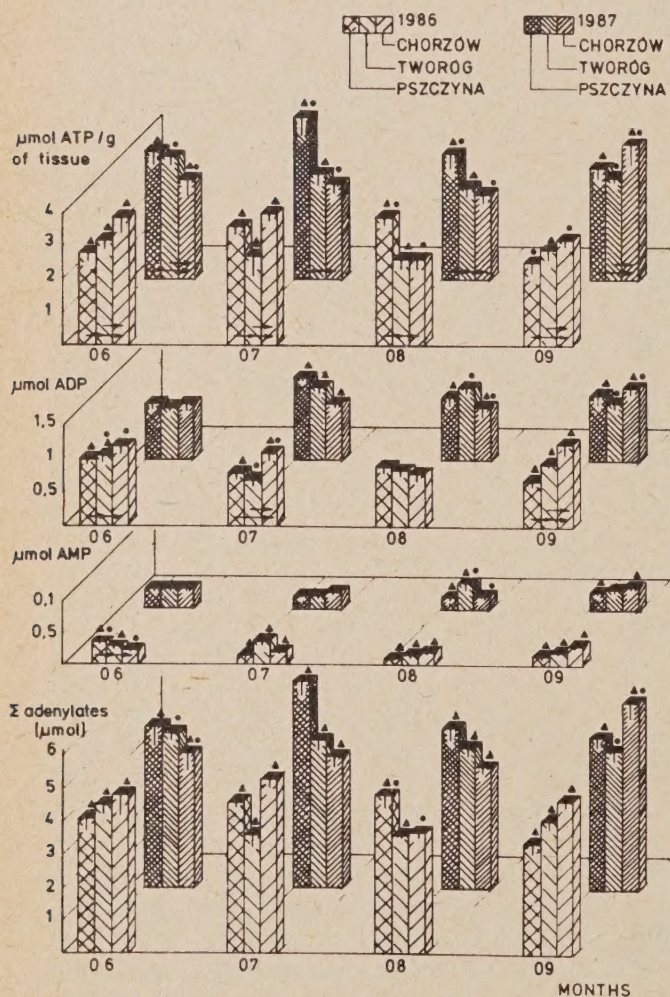


Fig. 5. Changes in the concentration of adenine nucleotides in the body of honey bees collected from 3 sites in the Upper Silesia. Monthly averages in μmol with S. D. Means marked with the same figure are significantly different.

trophic components. It forced us therefore to include an ecophysiological approach to this study. More accurate prediction of acceptable levels of xenobiotics was thus possible by measurements of nonspecific indicators: the adenylate energy charge and total pool of adenylates (HAYA, WAIWOOD, 1983).

An average life span of a foraging honey bee is about 30 days (BŁASZCZYK, 1987). Measurements of their body burdens with heavy metals showed a great variability within the season. These data reflect to what extent the action of pollutants on foraging insects is modified during the season by overall environmental factors (wind direction, rain fall, temperature and others). These factors have a special importance for organisms residuing in locations situated on the border of main pollutant sources, eg. Tworóg and Tworóg-Koty. General analysis and forecasts may be more precise on the basis of annual averages (Fig. 2). Recent data confirmed the positive correlation between contents of metals in insects and in their habitats (Fig. 2), but, as this study is in progress, final conclusions will be outlined later.

The highest correlation between metal content in the body and environment was found for zinc, and to less extent for Cu, Cd and Pb. It is supposed that observed differences reflected variations in mobility of these metals in the environment. Zinc is easily absorbed by plants

from the soil, both actively and passively, thus the concentration of Zn in plants rises progressively with its content in environment (KABA-TA-PENDIAS, PENDIAS, 1979). Elevated body burdens with zinc were also found in many insects from contaminated areas (HUGHES et al., 1980; MARTIN et al., 1982; HUNTER et al., 1987a). GRODZIŃSKA et al. (1987), have proved quite intensive accumulation of zinc also in a grazing food chain. High concentration

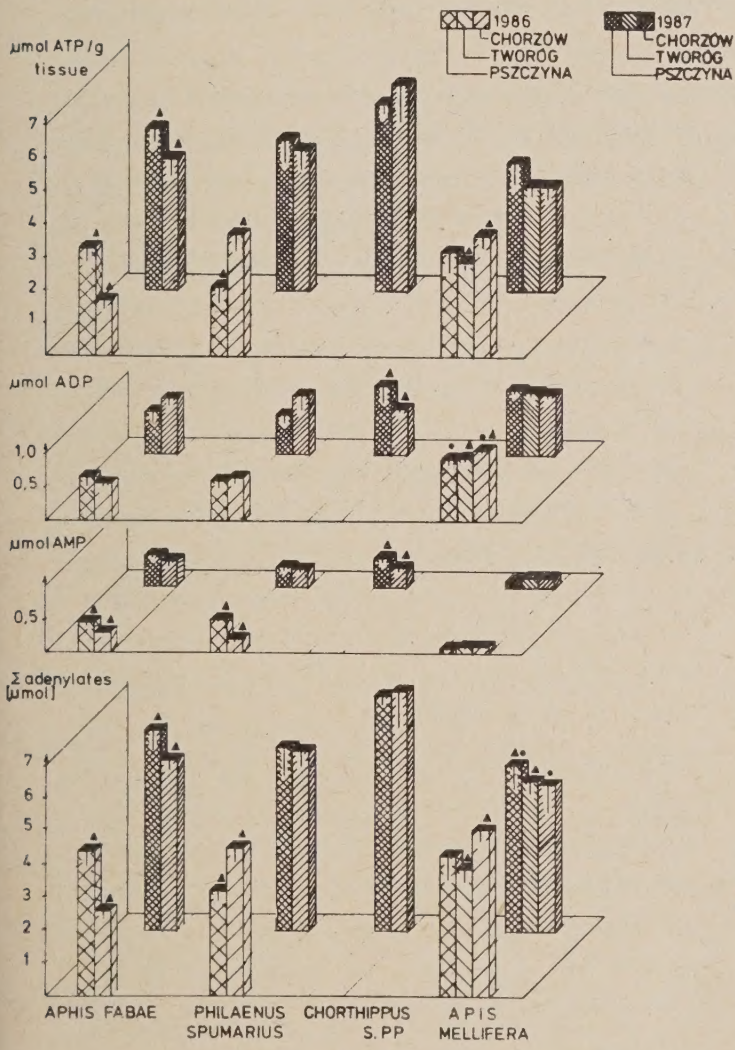


Fig. 6. Concentration of adenylate nucleotides in some insect species collected from various sites in the Upper Silesia. Annual averages in μmol with the same figure are significantly different.

of zinc have also been reported for honey bees (JONES, 1987). Copper is an essential metal but is potentially toxic. Bioaccumulation of zinc in invertebrates is usually high, but in some species the level of copper remains steady at least for certain range of its external concentrations (BEYER et al., 1982; JOOSSE, VERHOEF, 1986; AMIARD et al., 1987). Variations in concentration of Cu in honey bees from Silesian Region reflected overall levels in the polluted environment (Fig. 2). HUNTER et al. (1987) found that seasonal pattern of accumulation of Cu and Cd in *Chorthippus brunneus* closely followed seasonal trends in metal contamination levels in vegetation, but 85 % of total body copper and cadmium is associated with the integument. BROMENSHENK et al. (1985) reported, however, that Cu Zn and Pb in bee tissues could not be related to the environmental distribution of these metals. Lead exhibits the lowest mobility among analysed metals, thus it probably reaches bees in flight, from contaminated air or from dust that may have settle out on contacted surfaces. TONG et al., (1975), had reported that bees often mistake dusty surfaces or various finely powdered materials outdoors for pollen and can carry them to the hive. Partial correlation between Pb concentration in bees and the air confirmed these statements, but body levels of Pb could not be related to the data on the dust-fall occurring at the sample locations. Differences in metal concentrations are also related to properties of honey bees to discriminate between plants of different quality, varying mechanisms of storage and excretion of the metals, interactions within metals and availability of calcium (BEEBY, 1978; JOOSSE, VERHOEF, 1986).

Distribution of metal burdens in particular organs or tissues lead to conclusion that metal content in the gut appeared to be the better indicator of environmental contamination than the whole body burden. On the other hand, many factors detract from the reliable use of honey as

a bioindicator. Similar assumptions had been given by JONES (1987). He concluded that low metal concentrations in honey and their inherent variability detract the reliable use of honey as a monitoring tool. Observed variations in the metal contents of honey are also related to types of honey and their pH, flowers visited, size of foraging areas and others (FREE et al., 1983; JONES 1987).

Among many factors influencing the amount of metal content in pollen, the most important are the selectivity of a certain plant to accumulate metals and their concentration in the soil (FREE et al., 1983). In general, species differences in the mineral content of plants are reflected in the pollen, but pollen is usually collected by honey bees from a restricted group of plant species. Our results indicate that pollen may be used more profitably than honey as quality criteria in biomonitoring studies (Fig. 4).

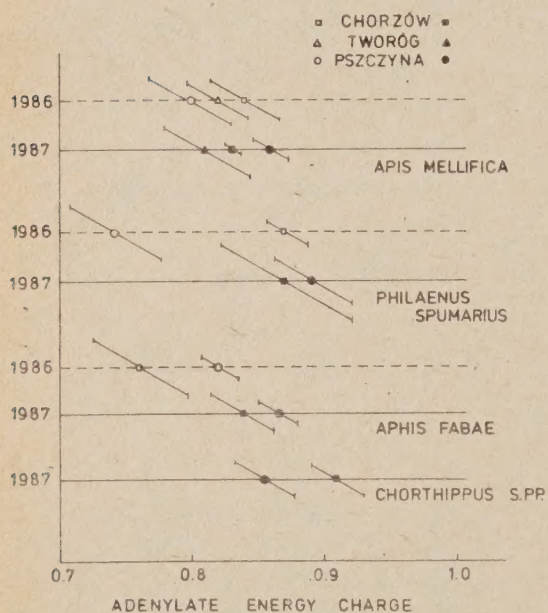


Fig. 7. The Adenylate Energy Charge in some insect species collected from various sites in the Upper Silesia.

This study was conducted to determine if the values of AEC and data on adenylate pool might be used in assessing the hazard potential of xenobiotics and as an early warning signals of energetic disruption in organisms exposed to various pollutants. It is a general measure of both indirect and direct impact of deleterious factors on the metabolic energy state (HAYA, WAIWOOD, 1983). The highest values for both AEC and adenylate pool were indicated for honey bees and sap feeding insects from less polluted site (Pszczyna). High levels of metals accumulated by insects from Chorzów have resulted in decreased energetic states, but within the range which is tolerated by the organism (HAYA et al., 1983). Lack of significant correlations between metal levels and changes in AEC and adenine nucleotide pool did not discriminate the usefulness of these biochemical criteria. It rather means, that metabolic functions of insects living within the study area are unaffected significantly by present levels of heavy metals and other pollutants.

C O N C L U S I O N S

1. According to increasing levels of environmental pollution sites selected to our study may be ranked in the following order: Pszczyna, Tworóg, Jaworzno, Tworóg-Koty, Chorzów, Ruda Śląska. Only a partial correlation was stated between contents of heavy metals in the air and in the body of honey bees.
2. Prevailing part of heavy metals burden is cumulated in the digestive tract of honey bees. Thus, measurements in this organ better reflect overall levels of metal accumulation that may occur in the polluted environment than metal contents in the whole body.
3. Low metal concentrations in honey and their inherent variability detract from its reliable use as a monitoring tool.
4. Pollen may be used more profitably than honey as a quality criterion in biomonitoring studies.
5. The values of Adenylate Energy Charge and pool of adenylate nucleotides may also be used successfully in assessing the hazard potential of xenobiotics, as an early warning signal of energetic disruption in organisms exposed to environmental stress. Highest values for both AEC and the adenylate pool were indicated for honey bees and sap-feeders from less polluted areas. Decreased energetic states followed high contents of metals in insects from more severely polluted areas (eg. Chorzów) but within a range which is still tolerated by the organisms.

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Bioindication of air pollution from nickel refinery dump

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Airborne metal particles in the vicinity of a nickel refinery arise primarily from the works, and secondarily from the waste dump. The waste contains a variety of metals that under some meteorological conditions create a metal particles aerosol influencing the ambient air adversely.

Because of little information on the effects of inhaled metal particles on animal organism, a monitoring of the polluted air by metals originated from a nickel refinery dump on domestic rabbits living in that area had been done. Domestic rabbits were used in our previous works for the monitoring of air pollution by magnesite and cement emissions (REICHERTOVÁ et al., 1981, 1984). TATARUCH et ONDERSCHEKA (1981) published the findings on the metal accumulation in the body organs of European brown hare exposed to some type of emissions as indicator for environmental pollution. The similar investigations were done by MAŇKOVSKÁ et al. (1980).

The aim of our present work was to establish the relationship between the metal accumulation in body organs of the exposed animals and morphological abnormalities in tissues on the light and electron microscopy level. A comparison between the field and laboratory exposure of animals was done to precise the effect of the metal accumulation in the body organs.

MATERIAL AND METHODS

Field exposure conditions

A group of domestic rabbits (*Oryctolagus cuniculus*) was exposed respiratory to metal aerosol originated from a nickel refinery waste for 6 months continually. A biomonitoring station was built up ca 4 km from a nickel refinery dump. The control group of rabbits was maintained in a nonpolluted area.

Laboratory chamber exposure conditions

A group of laboratory Wistar rats was respiratory exposed to metal dust aerosol for a period of 6 months, 4 h/day, 5 days/week at the chamber dust concentration of $50 \text{ mg} \cdot \text{m}^{-3}$.

Chemical composition of metal waste (%)

Fe_2O_3 - 64.40, FeO - 13.26, Fe - 0.35, NiO - 0.36, Cr_2O_3 - 3.68, CaO - 3.35, SiO_2 - 7.10, CoO - 0.06, Al_2O_3 - 3.66, MgO - 1.67, P_2O_5 - 0.10, V_2O_5 - 0.02, TiO - 0.12, S - 0.08, Cu, Pb and Zn (thousandths of %). Size distribution of dust particles varied within a wide range with marked proportion of respirable fraction, as assessed by light microscopy.

Metal contents (Fe, Ni, Cr) determination in the specimens of animal body organs

A method of atomic absorption spectrophotometry was used. The detail procedure was published in our earlier work (REICHERTOVÁ et al., 1986).

Histological examination of lungs

Tissue section from lungs were fixed in neutral-buffered formalin, cut in parafin at $7.5 \text{ } \mu\text{m}$

and stained in hematoxylin and eosin. The histochemical staining for Ni examination was done by the method of Feigl (LILLI, 1969). The histological changes were assessed under an Amplival (Zeiss) light microscope. Sections of lungs of animals were studied morphometrically. The media of 25 muscular pulmonary arteries in each animal was measured and expressed as a percentage of the external diameter. An average ratio for the medial thickness in each animal was thus obtained by the method of WAGENVoORT et al. (1974).

Ultrastructural investigation of myocardium

Tissue sections from myocardium were processed for electron microscopy by the method described by the producers of DURCUPAN^R - medium ACM Fluka. Ultra thin sections were cut on an ultramicrotome TESLA BS-490 A, stained with lead citrate by the method of BENABLE et COGESHAL (1965), and examined in a Tesla BS 500 electron microscope.

R E S U L T S A N D D I S C U S S I O N

As shown in Tab. 1, in the group of rabbits that inhaled metal aerosol for 6 months under the environmental conditions, an increase of the amounts of metals was detected in comparison to the control group. The content of Fe increased in the liver, thigh muscle, kidneys and heart muscle. The elevated concentration of Cr was found in the liver, thigh muscle, kidneys and heart muscle. As for the Ni content, the increased accumulation in the lungs, liver, and heart muscle was seen.

The data in Tab. 2 demonstrate the results of analysis of metal concentrations in Wistar rats following the 5-months exposure to metal aerosol under the laboratory chamber exposure conditions. As indicated in Tab. 2, the average amount of Fe was increased in Wistar rats in the lungs, liver and thigh muscle. The Cr content was elevated in the lungs, liver and thigh muscle too, while the Ni content was increased in the lungs, liver, thigh muscle, as well as in the heart muscle. The findings pointed out the fact, that the chamber exposure resulted in the accumulation of the all metals tested in the lungs predominantly, while following the field exposure only the Ni content increased in the lungs significantly. It could be suggested that under the extreme lungs burden ($50 \text{ mg} \cdot \text{m}^{-3}$) the inhaled metal particles are deposited in the lungs in contrast to the environmental conditions.

The heart muscle was examined under the electron microscope for ultrastructural deviations in the group of the exposed Wistar rats exposed under the laboratory conditions. The following changes were shown: a vacuolisation of cellular sarcoplasm, an initial homogenisation of mitochondrial crista and a swollen tubular sarcoplasmic reticulum. In the intercellular space the presence of an accumulated particular matter was seen, evoked probably as a consequence of an interaction between metal particles and proteins. That crystal-shaped matter reminded of crystals of hemoglobin. The same formations were seen also in the interstitium nearby the capillary blood vessels as well as among the collagenous fibrils. Concerning the myofibrils, any deviations could be seen.

The histological examination of the lungs of rabbits exposed under the environmental conditions showed a marked thickening of media and narrowing of lumen of muscular pulmonary arteries. Additionally, new branches of muscular media of pulmonary arteries arose, probably developed from fibroblasts. In comparison to the exposed Wistar rats the similar picture was

Tab. 1. Metal contents ($\text{mg} \cdot \text{kg}^{-1}$) in organs of domestic rabbits exposed environmentally for 6 months. E - exposed group, C - control group; $^+ P < 0.05$, $^{++} P < 0.02$, $^{+++} P < 0.002$, $^{++++} P < 0.001$.

Organs	Group (10+10)	Fe		Cr		Ni	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Lungs	C	18.1	6.84	0.3	0.08	0.3	0.01
	E	16.8	2.89	0.3	0.09	0.6	0.14 $^{++++}$
Liver	C	200.0	58.80	1.6	0.54	0.4	0.05
	E	338.0	30.50 $^{+++}$	3.2	0.30 $^{++++}$	0.6	0.20 $^+$
Muscle	C	9.3	4.15	0.2	0.05	0.4	0.05
	E	11.4	1.98 $^+$	0.4	0.11 $^{++}$	0.4	0.07
Kidneys	C	63.2	19.70	0.5	0.01	0.7	0.13
	E	112.0	10.40 $^{++++}$	1.1	0.10 $^{++++}$	0.6	0.12
Heart	C	38.4	8.53	0.4	0.01	0.3	0.07
	E	44.6	30.80 $^+$	0.6	0.01 $^{++++}$	0.6	0.07 $^{++++}$

Tab. 2. Metal contents ($\text{mg} \cdot \text{kg}^{-1}$) in organs of Wistar rats exposed to metal dust aerosol in the chamber at the dust concentrations of $50 \text{ mg} \cdot \text{m}^{-3}$ for 6 months. E - exposed, C - control; $^+ P < 0.05$, $^{++} P < 0.01$, $^{+++} P < 0.001$.

Organs	Group (10+10)	Fe		Cr		Ni	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Lungs	E	1000	200 $^{+++}$	21.0	2.2 $^{+++}$	144	36 $^{+++}$
	C	207	130	0.6	0.2	2	1
Liver	E	378	72 $^{++}$	0.8	0.1 $^{+++}$	4.1	0.7 $^{+++}$
	C	233	10	0.6	0.1	2.4	0.4
Muscle	E	26	1.5 $^{++}$	0.6	0.1 $^+$	0.7	0.2 $^{++}$
	C	22	3.7	0.5	0.1	0.5	0.2
Heart	E	160	15	0.3	0.1 $^{++}$	0.6	0.2
	C	176	17	0.2	0.1	0.6	0.1

seen, but the changes on pulmonary arteries were much less exposed than in rabbits. Using of the morphometrical method the thickening of media was assessed and compared between the control and exposed groups of animals. In the control groups the average ratio for medial thickness averaged about 30 % while thickening in the exposed groups ranged from 35 - 37 %.

Following the histochemical staining of lung sections for nickel an increased deposition of Ni in the walls of pulmonary vessels of the exposed animals was observed.

The ultrastructural deviations in tissue of myocardium as well as the thickening of muscular media of pulmonary arteries pointed out the possible danger of metal accumulation in the lungs and heart for cardiovascular system.

CONCLUSIONS

Waste from nickel smelter and refinery works contains a variety of metals, mainly Fe, Ni and Cr. Under some meteorological conditions arises a metal particles aerosol derived from nickel refinery dump, influencing ambient air adversely. Following respiratory exposure of a group of domestic rabbits (*Oryctolagus cuniculus*) nearby a nickel refinery waste dump a statistically significantly increased bioaccumulation of metals in the body organs in comparison to the control rabbits was found. Simultaneously, a laboratory chamber exposure of rats to metal aerosol for 6 months (4 h per day, 5 days per week) at the concentration of $50 \text{ mg} \cdot \text{m}^{-3}$ was performed. The preferable organs for the metal accumulation in that case were lungs and heart muscle.

The increased content of metals in the heart muscle evoked the ultrastructural deviations as investigated by the electron microscopy method. In addition, an accumulation of hemoglobin-like crystal-shaped matter was seen in the intercellular space. Using the morphometrical method under a light microscope a hypertrophy of media of muscular pulmonary arteries, especially expressed in rabbits exposed environmentally, was shown.

Our findings revealed the possible danger of the lung and heart burden by metal particles for cardiovascular system.

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Heavy metals in hair of hoofed game from Beskydy Mountains and their submontane area (Czechoslovakia)

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INTRODUCTION

Hoofed game can serve as an important bioindicator of environmental pollution of large regions. The important role is played by contamination of environment by immissions of industrial origin but on the other hand also by specific influence of biotope. Heavy metals concentrations in hair of hoofed game are suitable for monitoring of the impact of pollution on wildlife but other factors should be taken into consideration too. Application of statistical multispace analysis of these biogeochemical data contributes effectively to their analysis and understanding.

MATERIAL AND METHODS

Hair of 30 shot individuals of roe deer - Capreolus capreolus and red deer - Cervus elaphus was sampled from the neck of animals. Localities for this study were selected as a representative for region of Beskydy Mountains and two neighbouring regions: submontane hilly area and lowland of Ostrava - Karviná industrial region at Northern Moravia, Czechoslovakia. Ostrava-Karviná industrial area is highly polluted by coal mining and coal processing, metallurgy, chemical industry and electricity power plants. Beskydy Mts. are located SE of Ostrava in the distance of 30 km. The highest point Lysá Mt. reaches 1323 m above sea level. Transitional submontane region is a hilly area with average height of c. 600 m a. s. l. The degree of afforestation is highest in Beskydy Mts. (c. 90 %) and lowest in Ostrava - Karviná industrial area (c. 20 %) where flat lowland has average c. 300 m a. s. l. Climatic conditions differ accordingly to the height a. s. l. with highest precipitation and lowest mean annual temperature in mountainous area.

Samples of hair were washed two times in acetone and distilled water according to the standardized recommended procedure of International Atomic Energy Agency proposed for treatment of human hair. Dried samples were powdered in liquid nitrogen and prepared for analysis in the form of pellet by pressing by means of a hydraulic press. Chemical analyses were performed by energy-dispersive X-ray fluorescence spectrometry. This technique limited analyzed chemical elements to Ca, Fe, Cu, Zn and Pb. In case of Pb only 4 samples had concentrations higher than limit of detection, other elements were determined in all samples.

Analytical data were processed by cluster analysis and principal components analysis. This statistical multispace analysis was performed by set of computer programs developed by MACHEK (1988).

RESULTS AND DISCUSSION

The two clusters which were distinguished by cluster analysis can be characterized by arithmetical means for individual chemical elements listed in Tab. 1.

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Tab. 1. Concentrations of chemical elements (ppm) in hair of hofed game.

Cluster	CaO	Fe ₂ O ₃	Cu	Zn	Pb
I	1935	374	22	110	31
II	734	117	19	74	14
I + II	1294	237	20	91	27

Concentrations of chemical elements show some relationships. The most pronounced is the dependence Ca - Zn which is expressed by value of correlation coefficient 0.76. Significant values have also relationships Fe - Zn (0.44), Fe - Ca (0.44), Ca - Cu (0.32) and Fe - Cu (0.27). Correlation coefficient for relation Cu - Zn is the only one with very low value (0.07).

Concentrations found are in accordance with literature data (e. g. MAŇKOVSKÁ, 1980; TARUCH, ONDERSCHEKA, 1981).

The two distinguished clusters are very good represented in the plot of principal components (Fig. 1). Four vectors show role and significance of chemical elements Ca, Fe, Cu and Zn. This analysis allows to evaluate graphically the relationship between individual samples.

Samples belong to the four groups:

1. Surroundings of Lysá Mt., roe deer. Localities: Lysá Mt. (21), Smrk Mt. (22), Pražmo (24), Morávka (25, 26, 27, 28).
2. Mountain region of Jablunkov, red deer. Dolní Lomná (9), Horní Lomná (10, 11), Mosty (12), Tyra (13, 14), Kozubová (15).
3. Submontane area, red deer. Trojanovice (1, 2, 17, 18), Ondřejník (3), Mořkov (4, 5, 19), Veřovice (6, 7), Hukvaldy (8), Hodslevice (20).
4. Vicinity of Ostrava, roe deer. Šilheřovice (23), Louky (16), Bruzovice (29), Václavovice (30).

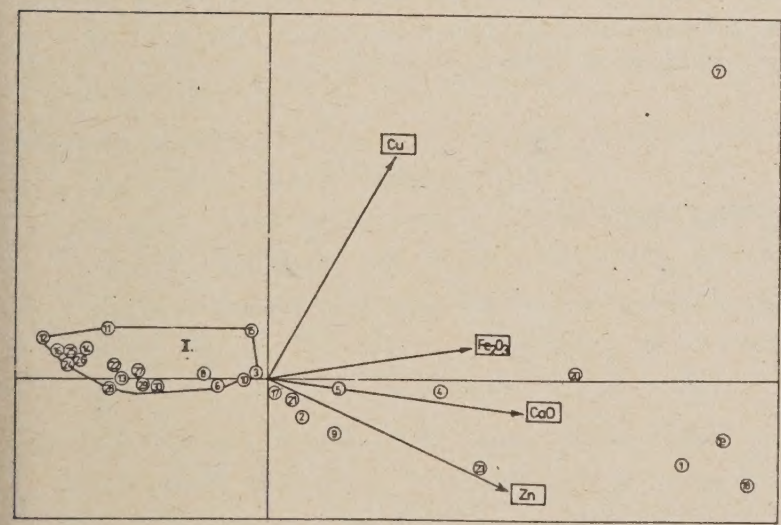


Fig. 1. Principal components plot - hair chemism of hoofed game (components 1 - 2).

Surprisingly enough, scatter fields in the plot for Capreolus capreolus and Cervus elaphus are overlapping. The distribution into two distinguished clusters is controlled rather by provenience from different types of localities. Experience gained by processing of unified data for roe deer and red deer shows that influence of different subspecies or populations inside certain species will be not significant for the application of hair chemism for monitoring of industrial pollution.

Samples from both mountain areas (localities of groups 1 and 2) are concentrated in the field of the cluster II. Most of samples from the vicinity of Ostrava belong also to this cluster. Samples from submontane area (cluster I) are widely scattered. Samples with high concentrations of Pb (21, 23 and 49) are especially anomalous. The cluster I and II are distinguished due to chemism variability of red deer hair. All samples of roe deer hair are concentrated in cluster II.

C O N C L U S I O N S

The results confirm the chemism of hoofed game hair depends on environmental conditions and it is possible to use it for an indication of industrial pollution in the study of large landscape regions. Individual animals are living in large but stable areas. Averaging and eliminating of local effects takes place which is suitable for large scale regional studies.

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Water plants as a bioindicator of radioactive pollution of surface water - application of a mathematical model

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INTRODUCTION

The pollution of surface water with radioactive liquid wastes from nuclear power plants during their normal operation is so small that a direct measurement of the concentrations of radionuclides in the water is too complicated due to their low and variable concentration values. In order to simplify the monitoring it is possible to use suitable bioindicators for the radioactive pollution. One of these is selected water plants especially for their significant capability to accumulate the radionuclides, for their relatively.

Models of uptake/release kinetics of radiocaesium in the system water - biomass of water plants have been created recently for non-rooting and submerged microphytic and macrophytic water plants (ŠVADLENKOVÁ, DVORÁK, 1987; ŠVADLENKOVÁ et al., 1987; ŠVADLENKOVÁ, 1987). In these models the reversible and irreversible sorption of radiocaesium by biomass and plant growth are studied. The water plants are considered there either as homogeneous one compartment or the plant biomass is divided hypothetically in two compartments with different kinetics of radiocaesium transport. The equation, or the system of equations describing this kinetics has then following form:

$$dx/dt = -p(t) \cdot x(t) + q(t) \cdot x_w(t); \quad x(t=0) = x_0, \quad (1)$$

resp.

$$dx_1/dt = -p(t) \cdot x_1(t) + q(t) \cdot x_w(t); \quad x_1(t=0) = x_{10} \quad (2)$$

$$dx_2/dt = \mu(t) \cdot x_1(t) - \mu(t) \cdot x_2(t); \quad x_2(t=0) = x_{20},$$

where x and x_1, x_2 are specific activities of radiocaesium in the biomass and in individual parts, resp., and x_w is the radioactive concentration of soluble form of radiocaesium in the water. The form of the time dependence of model parameters $p(t)$, $q(t)$ and $\mu(t)$ is following:

$$p(t) = K_1 + K_D + \mu(T(t), I(t)), \quad (3)$$

$$q(t) = (K_2 + K_D) / \alpha_1 + \psi^K(t) / x_w^K(t)$$

where K_1, K_2 and K_D (s^{-1}) are adsorption or diffusion transport coefficients that are considered in the model as time independent,

μ (s^{-1}) is the specific growth rate of algae that is the power function of temperature $T(t)$ ($^{\circ}C$) and of the light intensity $I(t)$ ($E \cdot m^{-2} \cdot s^{-1}$) in the water,

α_1 is a constant equalizing the part of dry and fresh plant mass,

$\psi^K(t)$ ($mg \cdot kg^{-1} \cdot s^{-1}$) is the specific rate of potassium uptake by algae and

$x_w^K(t)$ ($mg \cdot kg^{-1}$) is the potassium concentration in the water.

The form of time dependence of the model parameters and the estimations of parameter values are discussed in more detail in the study (ŠVADLENKOVÁ, M., 1987). In this paper the attention is paid to the possible use of the model (1) at the estimation of radioactive concentration

of ^{137}Cs in the water, $x_w(t)$, based on the knowledge of specific activity of plant biomass and the model parameters.

ESTIMATION OF RADIOCESIUM CONCENTRATION IN WATER

The solution of the Equation (1) for given initial condition has the following form:

$$x(t) = x_0 \cdot \exp \left[- \int_0^t p(s) \cdot ds \right] + \int_0^t \exp \left[- \int_s^t p(r) \cdot dr \right] \cdot q(s) \cdot x_w(s) \cdot ds, \quad (5)$$

Let us divide the time axis to intervals $I_i = \langle t_i; t_{i+1} \rangle$, $i = 0, 1, 2, \dots, n-1$ that always correspond to two successive sampling of water plants. The duration of these intervals is indicated with Δt_i . Suppose that in the interval I_i the values p , q , x_w are constant and equal to p_i , q_i , x_{w_i} . Denote $x(t_i) = x_i$ and $x_{i+1} - x_i = \Delta x_i$. Then - based on Eq. (3) - for the estimation of radiocesium in the water, x_{w_i} , in time interval I_i the following equation is valid:

$$x_{w_i} = \frac{p_i}{q_i} \cdot \left| \frac{\Delta x_i}{1 - \exp(-p_i \cdot \Delta t_i)} + x_i \right| \quad (6)$$

The precision of the x_{w_i} - estimation according to the Equation (6) was tested with help of the data set on specific activities of ^{137}Cs in filamentous algae Cladophora glomerata and the estimates of the other model parameters, p_i , q_i . The algae were sampled in the waste channel of the Jaslovské Bohunice nuclear power plant (Czechoslovakia). The water was sampled from the same site. These samples were analysed with gamma-spectrometric method. The maximum standart deviation for the measured values of specific activity of the plants and radioactive concentration of the water was 30 % and 10 %, respectively (VODRÁŽKOVÁ, SLÁVIK, 1985).

The mean values of the continuous functions $p(t)$ and $q(t)$ in the intervals I_i are taken as parameter values p_i and q_i .

RESULTS AND DISCUSSION

The estimation of radioactive concentration of ^{137}Cs - soluble forms in the water, x_{w_i} , calculated according to the Equation (6) is shown on the Fig. 1 (line). The actual values of this quantity (full circles) and of specific activity of ^{137}Cs in the dry substance of the algae Cladophora glomerata (blank circles) are also drawn on this figure. Between 20th and 60th days of the reference time more significant variations of specific activity of ^{137}Cs in the algae were observed. The estimation of x_{w_i} between two successive sampling of algae is here lower than the actual values of activity concentration of ^{137}Cs in the water.

If we do not take in account the first and the last experimental values of the concentration of ^{137}Cs in water (i. e. the 4th and the 169th days) then the mean relative deviation of the calculated value, x_{w_i} , from the actual value is 33,5 %.

Precision of the estimation of radionuclide concentration in water depends on the choice of time interval I_i magnitude between two successive algae sampling, on parameters magnitude and on the precision of their estimation and also on the discharging regime of radioactive liquid waste from nuclear power station. For optimal choice of I_i the magnitude of kinetic parameter $p(t)$ is taken in account. It is clear that for the duration of the time period Δt_i following relation will be valid:

$$\Delta t_i \sim \eta / p_i \quad (7)$$

We choose the magnitude of the parameter η according to the discharging regime of radioactive fluid waste. The smaller the time changes of specific activities of algae will be, the greater the parameter η will be. Actual values of p_i depend on the conditions on the given locality, e. g. the water plant sort, mean chemical composition of the water, light intensity and temperature in the water and vegetation season, etc.

Sensitivity analysis of the model showed that the model is sensitive especially to the change of the potassium concentration in the water and of diffusion transport coefficient. The precision of the x - estimation will increase by defining these two but also other (e. g. the specific growth rate of the algae and the specific rate of potassium uptake by the algae) model parameters more precisely.

CONCLUSION

With help of the model (1) it is possible to define the temporal course of radioactive concentration of the soluble forms of ^{137}Cs in the water with mean relative deviation 33.5 % namely based on the knowledge of the specific activity of the algae Cladophora glomerata samples and of the other model parameters. Model underestimates the actual values of the radiocesium concentration in water at vigorous changes of specific activity of the algae (by 2 - 3 orders during some days). The estimation can be defined more precisely by precisioning of the model parameters and by optimizing of the time period selection between two successive water plant sampling.

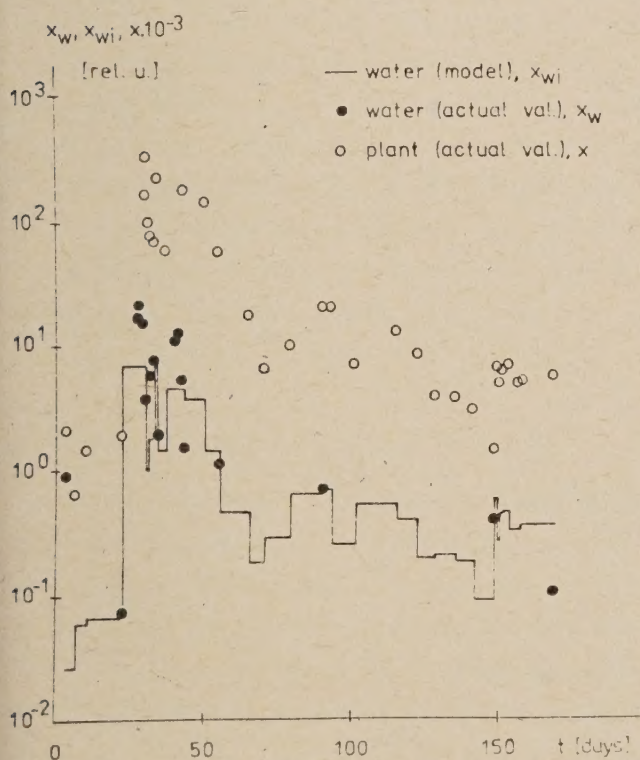


Fig. 1. The estimation of radioactive concentration of ^{137}Cs soluble forms in the water, x_{wi} (line) and the actual values of this quantity, x_w (full circles) and of specific activity of ^{137}Cs in the dry substance of the algae Cladophora glomerata, x , (blank circles).

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Influence of pollution with uranium and radium on population of *Vicia cracca* L.

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The interest in study of the influence of chronical irradiation on organism populations in natural ecosystems continuously increases (CHADWICK, LEENHOUTS, 1981; SCHEVCHENKO, 1987; and others). It is also caused, besides other, by quick development of atomic industry accompanied with pollution of some territories with uranium industry waste. Present time and future of such territories are serious ecological problems. Plants play an important role in protection of environment polluted with radionuclides as well by recultivation on polluted areas. The aim of our researches was to investigate the influence of pollution with uranium and radium on natural population of *Vicia cracca* L. that was dominant species of plant community growing over the polluted areas.

MATERIAL AND METHODS

Experimental territory was founded early in sixties. The surface of locality with increased ^{238}U content and products of its dissintegration was inactivated with a layer of sand of thickness 50 - 80 cm. This layer decreased the radiation over the soil surface and its penetration to the environment. But how the investigations lasting many years showed, water and wind erosions gradually decreased the sand layer and the radiation began gradually to increase. The plants that stepwise began to overgrow the impaired territory seemed to enhance the radiation.

In the time when we began our researches the territory was 20 years old and the perennial plant *Vicia cracca* become dominant species in plant community. This species was the main object of our investigations. We carried our radiochemical analysis of overground plant portions. We compared aberrant ana-telophases in root meristem of germinating seeds in control population and in population from polluted territory. We irradiated part of seeds from polluted territory before germination in order to determine their sensibility. We stated mutation frequency of chlorophyll lack in 8 population groups from polluted territory and compared it with this one in control groups. Further we followed the plant mortality set on loaded and on control territories. As criteria of health state of plants used following characteristics: seeds weight, part of sterile seeds, root and sprouts lengths of germinating seeds and the portion of nanistic individuals.

RESULTS AND DISCUSSION

Radiochemical analysis of overground plants (their parts) (tab. 1) showed that all tissues are considerably enriched with radionuclides at the end of vegetation period, especially with ^{226}U , the content of which was in stalks and in leaves three times higher than in control plants from unloaded territory. Calculations enabled to determine that during vegetation period (100 days) total load by incorporated radionuclides in overground phytomasis was 5 - 7 BER and in roots 50 - 70 BER. If we have taken in account even external irradiation of plants

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Tab. 1. Mean concentration of natural radionuclides in fertile plants V. cracca

Analysed plant part	^{238}U g/g dry matter $\cdot 10^{-7}$		^{226}Ra Bq/g dry matter		^{210}Po g/g dry matter $\cdot 10^{-3}$	
	control	experiment	control	experiment	control	experiment
leaves	0.15	4.0	0.26	395.9	44.8	625.3
stalks	0.09	3.8	0.26	333.0	17.4	403.3
legumes	0.02	0.2	0.44	51.8	3.0	10.7
seeds	-	-	0.52	20.4	4.8	3.0

with gamma-radiation the intensity of which was 100 times greater than in control locality so that we could assume the speed increase of mutagenic process in population of Vicia cracca on polluted territory.

A comparison of aberrant ana-telophases in root meristem of seeds from polluted and control populations collected in two series showed that polluted seed population exceeded the control population with account of damaged chromosoms (Tab. 2).

Supplementary irradiation of seeds before germination clearly showed that seeds of Vicia cracca from chronic irradiated territory were more sensible to irradiation than the seeds from control territory (Fig. 1). This was especially significant with doses of 90 - 110 Gr when the differences between frequencies of chromosomal aberrations were greatest.

Observation of mutation number of chlorophyll absence in population groups V. cracca from chronic irradiated territory showed that all population groups had lower frequency of pigment mutation than population groups from control unloaded area (Tab. 3). In our opinion this fact

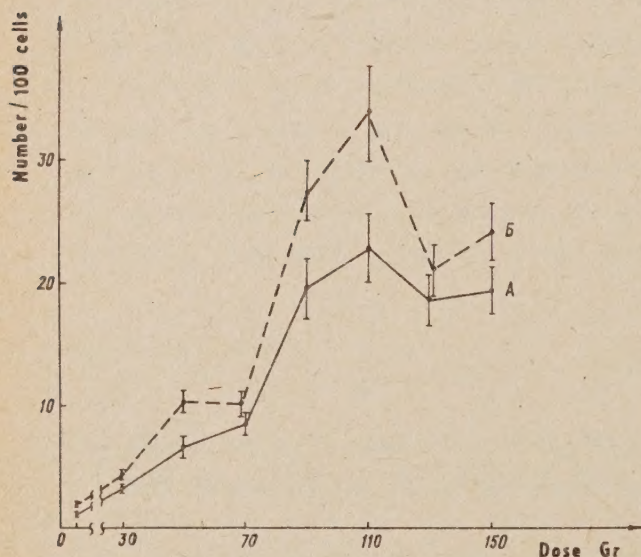


Fig. 1. Number of chromosomal aberrations in roots of germinating seeds from control (A) and polluted (B) territory irradiated with dose of 30 - 150 Gr.

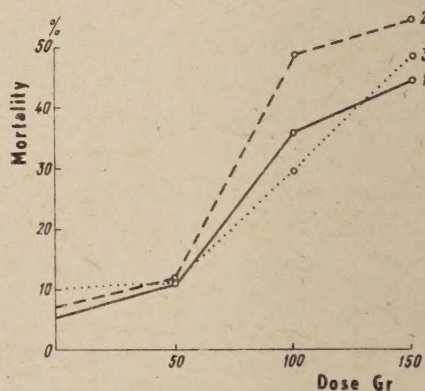


Fig. 2. Mortality curves of Vicia cracca on control area (1) and on area polluted with radionuclides.

Tab. 2. Chromosom aberation in mitotic cells of *Vicia cracca* roots in different radioecological growth condition. T - probability criterium of difference of small portions.
 + - $P < 0.001$.

	Number of ana-telophase cells with aberation, in %					
	bridges	fragments	bridges with fragments	retarde chro-mosoms	other ano-malies	total im-pairment
	Control (number of investigated roots - 144, of ana-telophases - 13234)					
series 1	0.470	0.425	0.085	0.076	0.021	1.114
series 2	0.803	0.750	0.304	0.383	0.185	1.303
average	0.064	0.064	0.195	0.023	0.103	1.209
	Chronical irradiation (number of investigated roots - 132, of ana-telophases - 13809)					
series 1	0.612	1.108	0.055	0.123	0.005	1.903
series 2	0.973	1.797	0.311	0.435	0.053	2.082
average	0.793	1.453	0.183	0.279	0.029	1.993
t	1.64	6.65 ⁺	0.90	1.131	1.56	5.42 ⁺

Tab. 3. Frequency of chlorophyll mutations in control and chronically irradiated population groups of *Vicia cracca*

Population group		exposition dose intensity, pA/kg	Number of fol-lowed sprouts	Number of mutant sprouts	Frequency of chlo-rophyll mutations %
Control	1	1.3	2174	105	4.82
	2	1.3	1948	89	4.57
	3	1.3	3375	76	2.25
	4	1.3	6330	115	1.82
Total:			13827	385	2.78
Chronicaly irradiated	1	180	7580	82	1.08
	2	36.0	3210	28	0.87
	3	10.8	5568	25	0.45
	4	25.6	5253	17	0.32
	5	14.4	4904	14	0.28
	6	14.4	4791	5	0.10
	7	28.8	6858	4	0.06
	8	94.0	2501	1	0.04
Total:			40665	176	0.43

could be explained to that factor complex characterising loaded area induced not only mutagenic changes but it influenced the elimination of some of them as well.

Investigations of the mortality of plant *Vicia cracca* on area polluted with radionuclides confirmed preceding hypothesis. It was stated that the mortality was very high at plants from polluted territory. Half of germinating plants perished far before the end of vegetation period (Fig. 2). Thus we assumed that the investigations of sprouts for manifestation

of mutation of chlorophyll absence that we carried out generally early in the spring when the temperature strongly changed during the day the chlorophyll substance could not be stated due to great mortality of specimens with decreased vitality.

Investigation of mutation frequency and plant mortality proves that the state of Vicia cracca population is not normal on polluted territory. Great part of seeds that grow here is damaged. These seeds either do not give the offsprings (they perish in germination stage or this of young plants ones) or the offsprings are weakened. Other characteristics of plants from polluted territory differ also from the characteristics from plants from control territory. Mean weight of plants at the end of vegetation period from polluted territory is two times lower as the one of plants of control territory. Between the offsprings from polluted territory there exist a great deal of nanistic individuals (to 50 %). Lower weight of seeds was stated at plants from polluted territory (on the average by 20 - 25 %) and their higher sterility (by 26 - 33 %) in comparison with the control ones. The length of roots and sprouts of germinating seeds from polluted territory was 3 - 1.5 times smaller than at control plants. The number of grains (plant and number of legumes did not significantly differ statistically from polluted and control territory.

C O N C L U S I O N S

1. Comparison of different pollution criteria of Vicia cracca from chronically irradiated area and from control area showed lower vitality of the population from polluted area (seed weight and portion of sterile seeds, plant mortality, mean weight of plants, number of nanistic individuals, length of roots and sprouts).
2. Analysis of meristematic cells of seed sprouts showed increased portion of damaged chromosomes in ana-telophases of seeds from polluted area what proved higher rate of mutagenic process in chronically irradiated population.
3. Lower frequency of chlorophyll mutations was found in population from irradiated area and this proved that the complex of radioecologic conditions on polluted territory was not only be an inductor of new mutations but also an selection factor oriented towards the individuals with decreased vitality, e. g. towards the bearers of mutations blocking the synthesis of chlorophyll.
4. Higher sensitivity to radiation of seeds from polluted territory was found with help of criterium of chromosomal aberation in mitosis, evidently caused by changes in their genom.

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The use of basidiomycete fruiting bodies for bioindication of radionuclides

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INTRODUCTION

This paper reports the use of analyses of fungal fruiting bodies as a bioindicator method for investigating the local environment of nuclear power stations. General advantages and disadvantages of this method are apparent and it is necessary to bear these in mind from the start.

Advantages. The fungal fruiting bodies have a great accumulating ability and could therefore give a very easy indication of radioactivity increases in an investigated area. Therefore this method of radiometric analysis could be as useful as that of spruce needle analyses, which are used for monitoring around nuclear power stations, for example in Japan (MATSUOKA, MOMOSHIMA, 1983).

Disadvantages. The accumulation of radionuclides depends strongly on the species of fungi and physical and chemical characteristics of the site. Also since the aerial atom bomb tests of the 1950's and 1960's, radionuclides such as ^{137}Cs and ^{90}Sr are now present in almost all parts of the environment. Thus the additional contribution of radionuclides from the normal operation of nuclear power stations may be so small as to be hidden within the error term of the analytical method used.

Perhaps therefore, radiometric analyses of fungal fruiting bodies could be best used in baseline studies before a nuclear power station became operational, so as no doubts could exist to the origin of future potential atmospheric discharges.

In this work we deal with radioactivity measurements in fungi that increased as a consequence of the accident at the Chernobyl nuclear power station. Lack of the effective containment during that accident meant that very large size range of radionuclide particles was released but only the finest were subject to long-distance transport. We believe that should the maximum credible accident occur at a PWR-type station in this country e. g. Temelin, the pattern of deposition in the CSSR would be directly parallel to that produced by Chernobyl, since more effective containment would ensure that only the finest radionuclide particles could be released.

After the accident at Chernobyl, near Kiev in the Ukraine (26th April 1986) a relatively large amount of radioactivity was released, with its dispersion being controlled primarily by meteorological conditions prevailing at that time. Measurements carried out at different sites in Europe showed that the concentration of radionuclides deposited was particularly dependent on altitude (KOLB et al., 1986; PERRSON, RODHE, 1987). Deposition of radioactive element occurred in South Bohemia between the 1st and 10th May 1986 (Zpráva, 1987). In the mixture of radionuclides deposited, we considered the content of ^{137}Cs , ^{134}Cs and ^{103}Ru to be the most serious. The concentration of ^{90}Sr was quite low (Zpráva, 1987).

The pattern of contamination was uneven over to land surface; this heterogeneity was caused by a variety of factors, especially variation in the fall-out from the radioactive cloud,

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but as yet these differences are unexplained (APSIMON, SIMMS, 1986; KOLB et al., 1986; SPURNÝ et al., 1987; Summary, 1986). However differences in the transport mechanism are likely to explain the lower $^{134}\text{Cs}/^{137}\text{Cs}$ ratio found in all terrestrial samples when compared with atmospheric samples taken at the time. Levels of ^{103}Ru were at first easily measurable but with its short half-time (40 days), levels quickly dropped.

MATERIALS AND METHODS

Fungal fruiting bodies were collected at several locations in South Bohemia (Fig. 1) at different times during 1986 and 1987. A more representative sample set was obtained during the 1987 field work.

After identification the fruiting bodies were excised and firstly air-dried at laboratory temperature followed by oven-drying at 105°C until they reached constant weight. The fungi were not washed before drying to facilitate comparison with the procedures used at other laboratories. The dried fungi were then simply ground although some difficult samples were freeze-ground using liquid nitrogen. The crushed material was then put into 100 ml polyethylene flasks and measured with a scintillation tube used in conjunction with a multichannel analyser (Nuclear Data ND 65B) of which 256 channels were used. As the volume of the sample obtained varied greatly (10 - 80 ml) the complete sample was counted and then corrected using a calibration curve. Measurement time was from 15 minutes to 24 hours depending on sample activity. The relative error was less than 10 % for ^{134}Cs . All the measured concentration were expressed as a proportion of dry matter. Results were completed on ZX Spectrum (48K) using a Datalog programme.

RESULTS AND DISCUSSION

The concentration of ^{137}Cs and ^{134}Cs are shown in Tab. 1. Sample sizes were small and thus our results cannot be given statistical significance. However the following points may be made:



Fig. 1. Sample situation-South Bohemia
□ - Locality

Tab. 1. The concentration of ¹³⁷Cs and ¹³⁴Cs in fungal fruiting bodies.

Locality, Altitude, Sampling Date			
Species	Concentration Bq/g		
	¹³⁷ Cs	¹³⁴ Cs	¹³⁴ / ¹³⁷ Cs
Zliv-Blana, 390 m, 20.9.1986			
Boletus badius	6.65	2.09	0.31
Lactarius rufus	6.94	2.11	0.30
Lepista nuda	0.09	0.00	0.00
Pholiota aurivella	0.38	0.26	0.67
Pisek, 400 m, 16.11.1986			
Clitocybe-cap	0.25	0.09	0.36
- stalk	5.14	2.07	0.40
Coprinus comatus-body	0.90	0.32	0.36
-hyphae	4.00	1.40	0.35
Psathyrella-cap	0.41	0.06	0.27
-stalk	0.21	0.06	0.27
Munice, 380 m, 30.9.1987			
Agaricus arvensis	0.21	0.00	0.00
Collybia dryophila	0.87	0.14	0.16
Collybia dryophila	0.64	0.10	0.16
Cortinarius anomalus	10.68	1.64	0.15
Cystoderma cinnabarium	1.04	0.49	0.47
Lactarius vellereus	0.29	0.07	0.23
Russula (mixture)	1.02	0.20	0.19
Russula vesca	0.29	0.12	0.40
Scleroderma citrinum	0.20	0.05	0.27
Chotýčany, 520 m, 18.8.1987			
Boletus aestivalis	0.62	0.00	0.00
Boletus chrysentereon	1.76	0.00	0.00
Leccinum scabrum	1.50	0.00	0.00
Lepiota rhacodes	0.86	0.00	0.00
Russula cyanoxantha	0.67	0.00	0.00
Chotýčany, 520 m, 25.10.1987			
Amanita muscaria	0.17	0.00	0.00
Clitocybe nebularis	0.13	0.06	0.45
Pholiota sp.	1.13	0.95	0.42
Horská Kvilda, 1100 m, 10.10.1987			
Amanita muscaria	4.93	1.09	0.00
Boletus badius	57.60	11.15	0.19
Russula vesca	21.65	4.66	0.21
Stolová hora, 1200 m, 23.9.1987			
Boletus badius	63.70	13.94	0.20
Boletus chrysentereon	56.66	13.60	0.24
Boubín, 1300 m, 23.9.1987			
Boletus badius	140.53	30.46	0.22
Dermocybe crocea	81.28	19.10	0.23
Paxillus involutus	14.94	3.91	0.26
Russula ochroleuca	20.50	4.14	0.20
Boubín, 1300 m, 14.10.1987			
Paxillus involutus	37.14	8.04	0.22

1. In the same area examined (South Bohemia) an altitude-dependent gradient of ^{134}Cs activity was found to exist in fungal fruiting bodies; concentrations were also linked with landscape configuration.
2. In one location (Chotýčany) no ^{134}Cs was found in some of the fungi, demonstrating the heterogeneous nature of contamination.
3. The caesium accumulating ability of three fungi, Boletus badius, Boletus chrysenteron and Cortinarius anomalus was particularly high when compared with the other species analysed.
4. If the sample set is rearranged according to the ratio of $^{134}\text{Cs}/^{137}\text{Cs}$ found in the fruiting bodies, then the largest values were found in saprotrophic species regardless of location or soil type.
5. Taking into account the results of other authors (AICHBERGER, HORAK, 1975; BRUNNERT, ZADRAŽIL, 1983; ECKL et al., 1986; ERNST, ROOI, 1987; GERZABEK et al., 1988; HASELWANDTER, 1977; MUTSCH, 1979; SEEGER, SCHVEINSHAUT, 1981; TEHERANI, 1987) we can state that the variation in caesium content of fungi is large, even within fungi of the same species. For biomonitoring purposes care should be taken to minimize these differences by selecting both control and contaminated sites with similar chemicals and physical characteristics. Differences in caesium content between fruiting bodies and hyphae are as yet unexplained.
6. With our current knowledge of radionuclide uptake (and particularly caesium) by fungi it is not possible to recommend the use of this simple sampling and analytical method for environmental monitoring of nuclear power stations. However the great accumulating ability of the fungi is a property that should not be overlooked and should be elaborated further. But at present level of knowledge does not allow an unambiguous interpretation of our results, which is a stringent condition for monitoring.

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Observation of ^{131}I retention in bee-collected pollen

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Monitoring activities undertaken in our institute after Chernobyl accident covered among others semiconductor gamma-ray spectroscopy measurements of the contamination of the ground layer of the atmosphere in Prague as well as that of bee products collected at Dol u Prahy.

Both aerosols and bee products were collected from 2nd May to 12th May 1986 with a typical sampling period of 24 hours. Aerosol sampling method employed is thoroughly described in TOMÁŠEK et al. (1987). Briefly, it is based on forcing the air through a special fiber filter fixed in a sampling station.

Pollen was collected using perforated barrier, openings of which having 3.2 mm in diameter. Pollen-loaded bee when trying to get into its hive, loses a part of its pollen that falls off in a storage. Thirty milliliters of pollen was taken every day from the storage to represent that day's collection (TOMÁŠEK et al., 1988).

Both pollen- and aerosol samples were then put in a appropriate plastic bowl on the detector face (coaxial Ge-Li, 65 cm³) with a counting time up to 60 000 seconds in a fixed geometry (RYBÁČEK et al., 1987). The obtained trends of I-131 and Cs-137 activity concentration in pollen and ground layer of the atmosphere in Prague are given in Fig. 4 and Fig. 1 respectively.

Besides measurements mentioned superficial activity F (Bq/m²) of I-131 and Cs-137 in the region of Prague was calculated solving differential equation (1a), using ČSSR - averaged deposition rate v_g (mm/s) and Prague's atmospheric activity concentration data A (Bq/m³), corrected for decay to 0 hrs. GMT 26.4.1986 (λ is a decay constant.):

$$\frac{dF}{dt} + \lambda F = v_g \cdot A \cdot e^{-\lambda t} \quad (1a)$$

Further analysis of data on I-131 and Cs-137 activity concentration in pollen and of those on superficial activity (see "Explanation") showed that the concentration ratio $C_{\text{I-131}}/C_{\text{Cs-137}}$ for the examined pollen was a few times higher than was that for the fallout deposited on surrounding ground. Effective removal constants for ^{131}I and ^{137}Cs were derived from their concentration trends and it was stated that the exchange of ^{131}I in pollen was substantially faster than was that of ^{137}Cs .

EXPLANATION

Let us assume the first order kinetic of exchange between pollen and environment with input controlled by fallout rate F

$$\frac{dP_i}{dt} + (\lambda_i + \Delta_i) P_i = k_i F_i \quad (1b)$$

where λ and Δ are decay and removal constants, respectively. Neglecting $\dot{F}$ for $t > 5$ days, the ratio $R = P_{\text{I-131}}/P_{\text{Cs-137}}$ is given by

$$R = R_0 \cdot \exp(-r \cdot t) \quad (2)$$

where R_0 is a constant and

$$r = \lambda_{I-131} - \lambda_{Cs-137} + \Delta_{I-137} - \Delta_{Cs-137} \tag{3}$$
$$r = 0.086 + \Delta_{I-131} - \Delta_{Cs-137} \quad (\text{day}^{-1})$$

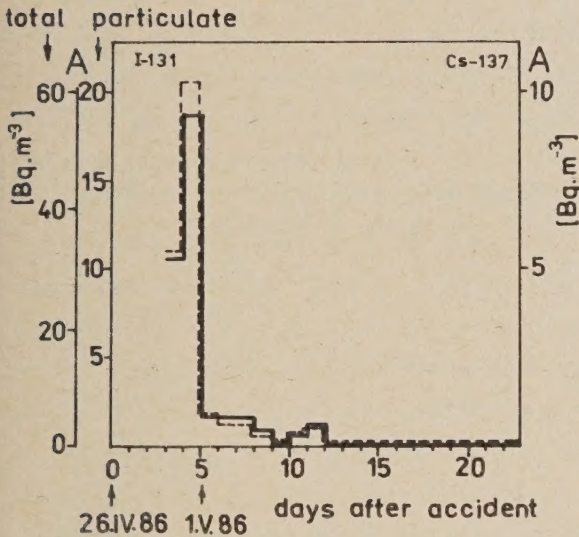


Fig. 1. Trend of atmospheric I-131 and Cs-137 activity concentration in Prague, A, after Chernobyl NPP accident (A is corrected for decay to 0 hrs. GMT 26.4.1986).

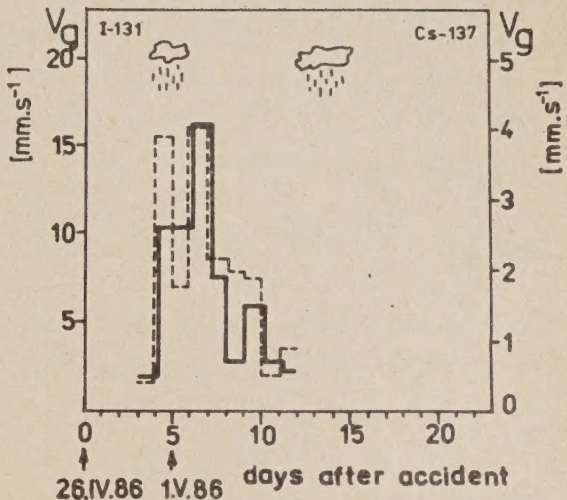


Fig. 2. Mean I-131 and Cs-137 deposition rate in CSSR, v_g (average from several sampling sites).

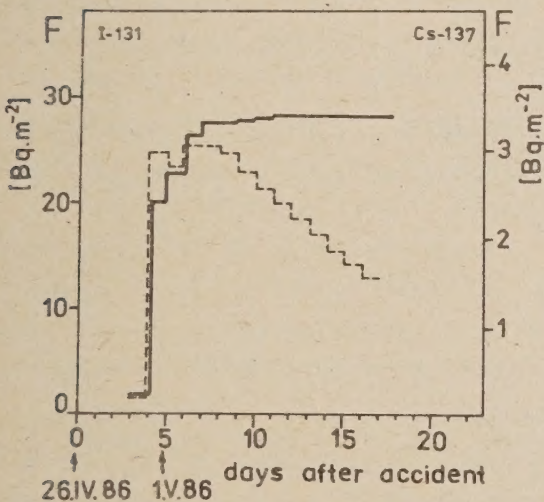


Fig. 3. Calculated trend of superficial activity F of I-131 and Cs-137 near Prague.

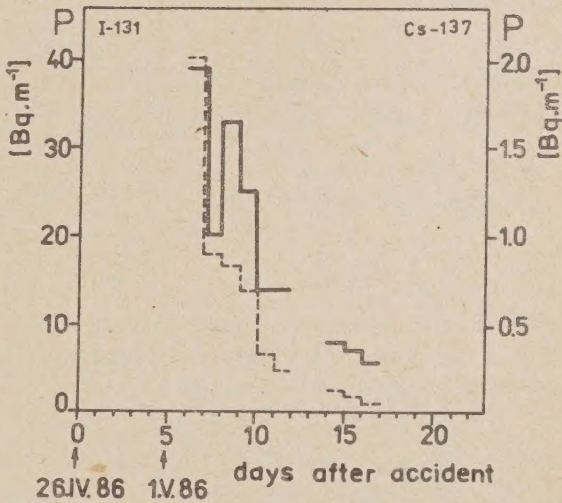


Fig. 4. Trend of I-131 and Cs-137 activities in pollen from Dol near Prague, P, after Chernobyl NPP accident (P is corrected for decay to the end of sampling period).

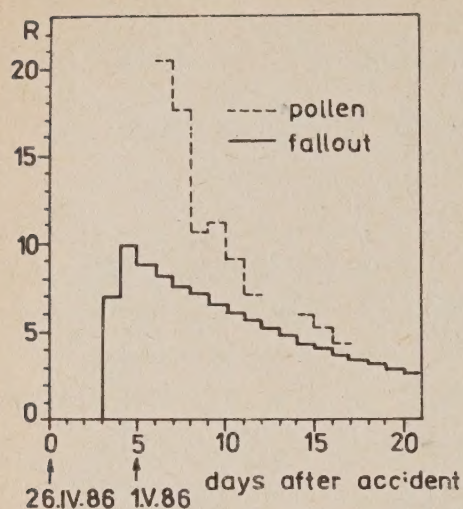


Fig. 5. Trend of activity ratio I-131 to Cs-137, R , in pollen and fallout (soil) (R is corrected for decay to the end of sampling period).

The least-squares estimates of $\ln R_0$ and r are $(3.70 \pm 0.38)_{0.95}$ and $(0.141 \pm 0.034)_{0.95}$, respectively. Let us test the hypothesis $\Delta_{I-131} = \Delta_{Cs-137}$ i.e.

$$H_0 : r = \lambda_{I-131} - \lambda_{Cs-137} ,$$

$$\bar{H} : r > \lambda_{I-131} - \lambda_{Cs-137}$$

by means of t-test:

$$t = \frac{0.141 - 0.086}{0.0144} = 3.83, \quad t_{0.95}(7) = 2.37$$

The value of t (determined with 7 degrees of freedom) indicates that H_0 has to be rejected even on the level 0.995 i. e. I-131 and Cs-137 removal rates differs significantly.

The exchange of I-131 between pollen and environment is thus more rapid than that for Cs-137. It results in higher ratio R for pollen compared to analogous ratio for fallout a few days after deposition (due to faster "capture" of I-131).

However, the ratio R falls down and finally has to be smaller than analogous ratio for fallout (due to faster removal of I-131). It is apparent that different rates Δ could explain observed behaviour of ratio R .

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Principles of using wild animals as bioindicators of global radioactive pollution

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The great attention paid to the environmental conditions has aroused interest in the problems of biological indication. Some problem of bioindication covers a complex of phenomena known to every-body as the "pollution". Radionuclides which readily incorporate into animal tissues and pass along food chains: ^3H , ^{14}C , ^{90}Sr , ^{131}I , ^{137}Cs are of great importance in pollution studies. In future I shall name ^{90}Sr and ^{137}Cs without atomic number for simplicity.

The ecologist's attention has always been attracted by the fission products of uranium and plutonium, particularly long-lived Sr-90 and Cs-137 found on the ground surface after nuclear weapon tests as well as after the processing of the uranium-containing ores and regeneration of the utilized nuclear fuel. Many radionuclides, including Sr and Cs were readily involved in the biogenic turnover. They can be accumulated by animals in detectable amounts.

Nuclear tests in the atmosphere and in the Earth surface have resulted in widespread distribution in the environment radioactive of fission and activation products. Last yield test programmes were conducted in 50'-s and in early 60'-s. In the last 20 years the radioactive fallout density was greatly decreased after signing the Moscow agreement banning nuclear tests in three media. However some countries (China and France) is continuing nuclear tests in atmosphere. To 1980 less than 2 % of all amount of Sr and Cs remained in the stratosphere. The remainder has been deposited on the Earth surface.

However underground nuclear and explosions with peaceful purposes are continuing up to date with partly release of radionuclides to atmosphere. This release may be significant. So yearly radioactive release due to underground nuclear tests in USA from 1963 to 1971 is equal to three atmospheric bomb explosion like Hiroshima one.

At present the problem of man-made radionuclides input into the environment acquires one more actual aspect. Sr and Cs make up not so small fraction in radioactive wastes produced in fission fuel process in nuclear installations.

The accidents that took place in the nuclear installations and atomic power stations resulted in the release of radionuclides to the environment too.

Maximum global radioactive fallout on the USSR territory was recorded in 1959 - 1963. In 1983 - 1985 the average of Sr content in the soil was from 3 to 9 GBq/km² and 4.5 to 15 GBq/km² for Cs.

The penetration of radionuclides into terrestrial animal's bodies occurs by means of the alimentary way: with food and water as well as via respiration of radioactive aerosols or gases. However radioactive penetration as absorption of polluted soil particles - swallowed by animals during cleaning their integuments should also be taken into account. In addition, radionuclides may penetrate in the terrestrial animal body via diffusion through integuments, particularly in case of amphibians and some soil-dwelling invertebrates. Accumulation of radioactive isotopes in animals is proportional to the pollution density of ecosystems with fission products, Sr and Cs in particular.

Investigating regularities of the accumulation of radionuclides in animals, it is necessary to find out species differences in the concentration of radioactive substances. These differences may be connected with physiological features of animals, their morphology or diet, behavior, peculiarities of the population's distribution in ecosystems etcetera.

The most intensive study of the Sr and Cs migration from the global fallout along the food chains, involving wild and domestic animals and leading to man, was performed in regions of the extreme North of the USSR. The necessity of these studies was determined by the radionuclide concentration in the links of the chain: lichen - deer - man, exceeding by 10 - 100 times those in the identical links of other chains and other regions. In this case the clearly defined seasonal variations of Cs concentration in muscles of deer were marked. In winter it was 15 - 18 times higher than in summer. A similar situation occurs in Alaska and Sweden.

Seasonal variations of Cs content were also noted in wild deer, moose and, inhabiting the central part of the USSR. In the muscle tissue samples obtained from red deer, roe deer and moose, the concentrations of Cs were higher by 3, 10 and 30-fold as compared with cows from the same region. Differences were also manifest in the Cs concentrations in the muscles of fox, wolf and deer, inhabiting the same regions. In carnivores the radionuclide content exceeded that in herbivores by 2 to 3 - fold.

Thus, radionuclide concentration increases as it migrates in the food chain if the concentration is calculated for the organs, critical for the given radionuclide. If the calculation is estimated for the non-critical organs, radionuclide passage is marked by a considerable decline of concentration.

It's shown, that Cs and Sr are accumulated more intensively by the animals, which inhabiting high mountain regions. This regions as Caucasus are characterized by significant precipitation with global fallout of Sr and Cs. Many studies are available reporting in detail on penetration of Cs and Sr into the vertebrates body of various species. Their distribution in the body, tissues etcetra. Most of these experiments were carried out on laboratory and farm animals. The investigations on wild animals in nature show that the radionuclide penetration and distribution in them are subjected to the same rules of distribution and migration in the body as those of the element-analogues.

When radionuclides migrate through tropic levels, their concentration in organs and tissues is affected by isotopic and non-isotopic carriers. In chemistry the term "carrier" implies a weighty amount of the element, followed by the "weightless" amount of the another element in chemical reactions. The isotopic carrier is a stable isotope of the given element, it's chemical properties are identical to its radioactive isotope (for example ^{31}P and ^{32}P). The non-isotopic carrier is a stable isotope or isotopes of a chemical analogue of the elements identical to the radionuclide by the group chemical properties only. For example, Ca is carrier in respect to Sr, or K - in respect to Cs.

The term "observed ratio" (OR) was introduced to establish the Sr/Ca or Cs/K ratio in the biological system and the ratio of the source from where there ions enter the biological system. It expresses overall discrimination characterizing the transport of these elements from source into the biological system.

The decrease of Sr and Cs concentrations in the relation to Ca and K is typical for the first trophic level of both invertebrates and vertebrate, but it is true for insectivorous vertebrates belonging to different classes. The relative concentrations of Sr, and in some cases Cs, increase in predatory invertebrates and necrophages. The observed relations between herbivorous and insectivorous mammals are close for different species despite their food both as for Sr-Ca and Cs-K.

In the highest links of the trophic chain of vertebrates Sr concentration relative to Ca concentration is decreased. But Cs concentration relative to K concentration is increased.

During the investigations of Sr and Cs biogenic migration originated from global fallout, soil, vegetation and animal samples were taken from the different geographical zones. In all regions the Cs concentration was higher in vertebrates and insects. The animals with skeleton containing different Ca amounts accumulated a greater part of Sr. Thus, such animals as millipeds, wood-lice, molluscs and vertebrates can be used as bioindicators of radioactive pollution in ecosystems with Sr. But it is advisable to use vertebrates as bioindicators of relative pollution in ecosystems with Cs.

The Sr content in the animal biomass from different geographical zone changes over a wide range. However, in humid zones, animals accumulate in biomass on an average of 0.006 - 0.016 % and in arid zones 0.00003 - 0.0006 % of the Sr supply in ecosystems.

The function of animals from various groups in the biochemical turnover of Sr is different. The presence of invertebrates such as millipeds, wood-lice, earthworms and molluscs in the zoocenosis structure defines their high share in the biogenic migration of a radioactive isotope of strontium. As average 75 - 87 % of Sr, accumulated in the whole zoomass of these zones, account for these animals.

In mixed forests the most important role in Sr migration is attributed to molluscs accumulating 55 % of this radionuclide, contained in all animals. Taking into account the long-lived of these animals, it is necessary to note their importance in Sr biogenic migration particularly in the ecosystems, where mollusc's density is very high.

In broad-leaved forests of Caucasus the greater amount of Sr is accumulated by millipeds (fam. Julidae), molluscs and earthworms. Totally 80 % of the radionuclide contained in the zoomass, accounts for these animals. Millipeds, which are scarcely eaten by other animals, can become an effective depot of Sr.

In the animal population of the forest-steppe oak-grove the main quantity of Sr (about 60 %) is accumulated by earthworms. The role of vertebrates in Sr accumulation is not so great. This makes up from 11 to 16 % of the total zoomass stock in humid zones.

Cs content in the zoomass is 0.003 - 0.005 % in humid zones and $0.2 - 4 \cdot 10^{-4}$ % of the total amount of radiocaesium in arid zones. Such low indices are mainly defined by high capacity of Cs, particularly in soils rich with clay particles and containing a lot of organic material.

In Cs migration the vertebrates are of great importance, and less degree the earthworms and insects. On the average, these are 56 to 92 % of Cs, accumulated in the whole biomass, in the animals mentioned above. Vertebrates and insects, dominating in arid zones, contain practically all Cs accumulated in the zoomass.

The fairly small role of animals in biogenic radionuclide migration along the trophic chain is mainly compensated by the burrowing animal's activities. Furthermore, radionuclide redistribution in the soil significantly affects their further life.

In the forest-steppe, Spalax microphthalmus is one of the active soil-burrowing animals. To estimate the participation of these animals in the processes of Sr redistribution in the soil, the radionuclide dispersion in the out-burrowed soil was investigated. As a result of the burrowing activities of this animal, Sr is evenly dispersed in the soil layer in the range of 0 - 30 cm, while the control to 70 % of radionuclide is concentrated in the upper 10 cm layer.

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The accumulation of Cs-137 in earthworms and its transport in soil by earthworms

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I N T R O D U C T I O N

The ability of earthworms to mix various substances into soil is well known (MACKAY et al., 1983; SPRINGETT, 1983). Recently it has arisen more interest, in what extent earthworms can mix radioactive substances, e. g. radioactive fall-off, into the soil, or enrich them in their body. Some studies of the dynamics of radioactive cesium-137 in earthworms have been made, but in these studies Cs-137 has been used merely as a tracer, interest has not been the movement of Cs-137 itself. When radioactive fall-offs are used in soil or erosion studies (KACHANOSKI, 1987), the effect of soil mixing by earthworms must be taken into account. From the environmental point of view it is important to know to what extent the predators of earthworms are in danger or getting Cs-137 from earthworms.

In this work the aim was to study the effectiveness of ecologically different earthworm species in soil mixing. It was also studied to what degree radioactive Cs-137 can accumulate in earthworms.

M A T E R I A L A N D M E T H O D S

Field experiment was carried out on fine sand soil in Jokioinen, southern Finland. Experimental design was randomized blocks with two factors (sludge application and crop plant) and four replicates. Half of the plots were treated in spring 1987 with 20 t dw./ha sewage sludge that contained 6944 mBq/g dw. Cs-137, the other plots were fertilized equally with mineral fertilizers. The crop plants were barley and spring wheat. The plots were 2.5 m wide and 15 m long.

Earthworm sampling was done on the 8th of September by the formalin method (BOUCHE, GARDNER, 1984). In both ends of the wheat plots two half square meter contagious samples were taken. Soil was sampled by taking ten times 3.14 cm² samples to 15 cm deep around the earthworm sampling sites and pooling them. In all 16 soil and 32 earthworm samples were taken. After sampling, earthworms were put in 4 % formalin solution. Later earthworms were determined to species and dried in 105 degrees celsius for 24 h. For the measurements of Cs-137 all earthworm samples from same plot were pooled and worms belonging to different ecological groups were separated (BOUCHE, 1977).

Pot experiments were carried out in 8 l pots, which were filled with heavy clay soil. On the top of the pots 98 g dw. sludge was applied which is the same rate as in field experiment. Ten *Aporrectodea caliginosa* (Sav.) or five *Lumbricus terrestris* (L.), or no earthworms were put in pots, four replicates were used. The pots were kept in 10 degree celsius in darkness, and they were watered to keep optimum moisture for earthworms. After eighteen weeks the experiment was terminated. Five 3.14 cm² soil samples that were divided vertically into four parts (0 - 5, 5 - 10, 10 - 15 and 15 - 20 cm), were taken from every pot and samples from the same pot were

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pooled separating the depths. After that the earthworms were picked out, but not enough earthworms were alive for further analysis. The soil samples were dried and the Cs-137 was measured.

The Cs-137 measurements were carried out by Wallac gamma-ray spectrometer (DECEM-GTL) in 15 ml plastic test tubes. Before measurements the material was homogenized. The masses of samples varied between 1 and 10 g and the measurement time was 4 h. The measurements were made under 661.6 keV peak owing to Cs-137. After background subtraction the data was expressed as counts per second per gram dry weight. The values were converted to milli bequerels by counting a Cs-137 standard source of 5 ml volume. No corrections for self-absorbance were done. The results may not give very accurate absolute concentrations of Cs-137, but they give comparable results for the different treatment.

RESULTS AND DISCUSSION

Four species of earthworms were found on plots: Aporrectodea caliginosa, A. rosea (Sav.), Lumbricus rubellus (Sav.) and L. terrestris. Aporrectodea species were considered to belong to the endogeic, L. rubellus epigéic and L. terrestris anécique group (BOUCHE, 1977). There was significant difference between treated and untreated plots in concentration of Cs-137 both in the soil and the epigéic earthworms, but no difference in the anécique earthworms (Tab. 1. and 2.).

Tab. 1. Analysis of variance table of Cs-137 concentrations for soil and earthworm samples. Contaminated sludge was added 20 t dw./ha in treated plots.

		df	Sign. F
Soil	Sludge	1	0.0001
	Rep	3	0.0010
	Sludge Rep	3	0.0001
	Error	38	-
Endogées	Sludge	1	0.0005
	Rep	3	0.0168
	Error	4	-
Anéciques	Sludge	1	0.9729
	Rep	3	0.6018
	Error	4	-

ANOVAs for pot experiments showed significant differences in the amounts of Cs-137 in the depth 5 - 10 cm (Tab. 3 and 4.). There the concentration was higher in pots with A. caliginosa, because this endogéic species mixes soil very efficiently. Some of the A. caliginosa specimens were in diapause when the experiment was terminated. In the end of the experiment 35 % of A. caliginosa and 78 % of L. terrestris were alive, the biomasses per pot with standard errors were 2.71 (0.81) and 4.10 (0.33) grams, respectively.

The field experiments showed clearly that endogéic earthworms concentrate radioactive Cs-137. It was not clear whether cesium was in the tissues of worms or just ingested with other food, because the worms were measured with gut contents. The data gives us the idea that endogéic

Tab. 2. The amount of Cs-137 in the soil and in earthworms. Means with the same letter are not significantly different (Duncan's test, 5 %).

	Treatment	n	Mean (mBq/g dw.)	
Soil	Sludge	23	105	A
	No-sludge	23	40	B
Endogées	Sludge	5	157	A
	No-sludge	4	44	B
Anécigues	Sludge	5	102	A
	No-sludge	4	98	A

Tab. 3. Significance of F-statistics in ANOVAs of Cs-137 contents in soil in different depths for pot experiment. Treatments were control, ten *Aporrectodea caliginosa* or five *Lumbricus terrestris* in pot.

		Depth (cm)			
		0.- 5	5 - 10	10 - 15	15 - 20
	df				
Treatment	2	0.2416	0.0023	0.7079	0.6733
Error	9	-	-	-	-

Tab. 4. Mean Cs-137 concentration in soil (mBq/g) in pot experiments. Treatments are same as in table 3. Means followed by same letter are not significantly different (Duncan's test, 5 %). Number of observations is four for all means.

Treatment	Depth (cm)			
	0 - 5	5 - 10	10 - 15	15 - 20
A. caliginosa	811 A	171 A	105 A	110 A
L. terrestris	567 A	127 B	100 A	93 A
Control	695 A	100 B	119 A	101 A

earthworms feed selectively on sludge, which has higher nutrient content than the soil. Also, we can see clear difference between feeding habits of endogeic and anecique groups. The latter group feeds on litter and so the sludge treatment had no effect on the concentrations during first year. It is probable that cesium was ingested (CROSSLEY et al., 1971), because no differences in concentration of anécique group was found, so the active ion intake through the body wall did not take place.

The pot experiment confirmed that particularly the endogéic earthworms have quite a large capacity to mix various surface applied substances into the soil. *A. caliginosa* had better ability to mix sludge into the soil than *L. terrestris*. This disagrees with the results by SPRINGETT (1983), who showed that endogéic species mix soil laterally and anécique vertically. This may have something to do with the absence of light, because positive phototropism of *L. terrestris* is shown in low light intensities.

USATSEV et al. (1986) studied the distribution of Sr-90 by earthworms. They obtained quite similar patterns to the ones in this experiment. This should be kept in mind when for example radioactive fall-off is used as a tracer in studies of soil development and erosion (KACHANOSKI,

1987). The mixing was greatest in the top ten centimeters, which could lead to bias in results if the action of earthworms is not counted. The mixing could be due the direct effect of earthworms or by making of holes in soil and the subsequent increase in the water infiltration and ion transportation.

The value of earthworms as bioindicators of soil pollution depends very strongly on the ecological characteristics of species. This study shows that grouping earthworms at least in ecological groups is necessary when reliable results are wanted.

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Earthworms as bioindicators of radioactive pollution

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Bioindication of soil radioactive pollution is one of the aims of ecological monitoring.

Earthworms (Lumbricidae) have relatively high sensitivity to heightened contents of radionuclids in the soil. This fact may be explained in the following way.

Firstly, radiosensitivity of initial stages of development lumbricids (coccons and youngth) sufficiently high ($LD_{50/30} = 1 - 3 \text{ kR}$) (KRIVOLUTSKY, 1987).

Secondly, the long duration of life enables earthworms to receive a high cumulative dose.

Thirdly intestinal epithelium is one of the most radiosensitive tissue of lumbricids, therefore its intimate contact with radionuclids, especially with high linear dense of ionisation, leads to fatal consequences.

The action of all this factors, result in the significant decreasing of earthworms population in localities with heightened radioactive background. (In spite of high radioresistence of mature earthworms $LD_{50/30} = 60 - 160 \text{ kR}$ (KRIVOLUTSKY, 1987).

Some difficulties are encountered while using earthworms as bioindicators of radioactive pollution.

In f. Lumbricidae polyploidy is recognised to be a widespread phenomen. Polyploid complexes occurs in the most common representatives of USSR lumbricofauna: Eisenia nordenskioldi and Nicodrilus caliginosus.

Diploid N. caliginosus f. typica inhabit zone of coniferousbroad - leaved forestrs, triploid N. caliginosus f. trapezoides most common in south part of the Soviet Union (VIKTOROV, 1987).

E. nordenskioldi represents an intricate polyploid complex with various ($2n, 4n, 6n, 8n$) races and very considerable morfological polymorfism. The races of this complex can be found in a vast variety of habitats from Eastern Europe to Far East, and from Taimir to Altai. Diploid E. nordenskioldi restricted to local area near Novosibirsk, where octoploid race simpatrically occurs. E. nordenskioldi with octoploid number of chromosome also inhabit the North of the Soviet Union and Moscow region. Tetraploid race was found near Voronezh. Hexaploid race inhabits Central Chernozem region (BULATOVA et al., 1984; PEREL, 1987; VIKTOROV, 1988).

All recently investigated populations of E. nordenskioldi, inhabited areal edge, comprise polyploid individuals. This fact may serve as confirmation of the opinion that polyploid forms have greater hardinessunder extreme ecological conditions and a better colonizing potential.

It is matter of common knowledge that polyploid races of single species may significantly differs one from another in its physiology and therefore in response to radiation stress. Though we must know comparable radiosensitivity of polyploids in each concrete case.

Minimization of competition in a lumbricid community is achieved by niche partitioning and interspecies differentiation in resources utilization (LEE, 1987). This is manifested in particular in vertical stratification of lumbricid species wich inhabit single biotop. Therefore, when bioindication investigations are carried out, it is necessary to know, how ecological separation of lumbricid species take place in each concrete case, because this factor

determines earthworms contact with radionuclids on different stages of its penetrating into the soil. It is necessary to note, that some species of lumbricid, as for example Lumbricus terrestris, Nicodrilus longus, belonging to ecological group of burrowers, may increase the speed of the last process.

Thus, it is quite obvious that the integrated investigations of ecology and genetics of earthworms the most widely-spread species of earthworms is necessary because the unresolved problems mentioned above can create certain difficulties when results of bioindication investigation are interpreted.

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Freilebende Wildtiere als Bioindikatoren der radioaktiven Belastung

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Freilebende Wildtiere werden sehr häufig als Bioindikatoren in der ökologischen Forschung herangezogen. Besonders geeignet scheinen sie als Bioindikatoren für die Schwermetallbelastung von Ökosystemen, wie in zahlreichen Arbeiten gezeigt werden konnte (z. B. FRANK, 1984; HECHT, 1984; TATARUCH, 1982, 1984; etc.).

Die regional sehr hohe radioaktive Kontamination des Österreichischen Bundesgebietes durch die nach dem Reaktorunfall in Tschernobyl, UdSSR, freigesetzten Emissionen bot uns die Gelegenheit, auch die Rolle der Wildtiere als Bioindikatoren der radioaktiven Kontamination zu überprüfen.

Dies war im Rahmen der von den Österreichischen Behörden durchgeführten Messungen der radioaktiven Belastung des Wildbrets möglich.

M A T E R I A L U N D M E T H O D E

Um jene Tierart zu finden, die die beste Eignung als Bioindikator aufweist, wurden Proben möglichst vieler Arten untersucht.

Im Jahr 1986 stand uns Material von insgesamt 278 Wildtieren zur Verfügung, und zwar von 165 Stück Rehwild, 13 Stück Rotwild, 24 Stück Gamswild, 17 Stück Schwarzwild, 21 Feldhasen, sowie von 15 Stück Auerwild. Weiters gelangten Proben von Damwild (1), Mufflons (4), Sikawild (4), Steinwild (1), Birkwild (4), Fasen (2), Wildkaninchen (3), Fuchs (1), Wildgans (2) und Wildente (1) zur Untersuchung.

Das Untersuchungsmaterial stammte grossteils von erlegten Tieren (183), in den übrigen Fällen von frisch totem Fallwild (darunter 37 Verkehrstopfer). Folgende Organe wurden entnommen: Muskulatur (Oberschenkel bzw. Brust bei Vögeln), Leber, Niere und Milz. In der ersten Untersuchungsperiode (bis 15. Juli) wurden auch die Schilddrüsen untersucht. In wenigen Fällen wurden auch Analysen von Panseninhalt, Enddarmkot, Herz, Lunge und Buglymphknoten durchgeführt.

Der sehr hohe Anteil an Rehwild am Probenmaterial (59,4 %) ist darauf zurückzuführen, dass in den meisten Österreichischen Bundesländern die Jagdzeit für Rehwild (Böcke und Schmalgeissen) am 16. Mai beginnt (übrige Bundesländer 1. Juni) und deshalb die Frage nach der Genusstauglichkeit des Wildbrets von Rehen vorrangig behandelt wurde.

Bis zur Messung der radioaktiven Kontamination wurden die Proben bei -20°C aufbewahrt.

Die Proben wurden manuell grob zerkleinert und in Standard Dosen zur Erzielung einer konstanten Messgeometrie gefüllt. Die Messung selbst erfolgte mittels hochauflösender Gammaskpektrometrie unter Verwendung von Ge(Li)-Halbleiterdetektoren. Die Spektrenauswertung wurde über eine Vielkanalanalysatoranlage von Canberra und einen Rechner PDP 11/34 durchgeführt, wobei kommerzielle Software verwendet wurde. Die Kalibrierung erfolgte mit einer Radionuklidmischung der Fa. Amersham in der jeweilig gleichen Messgeometrie. Durch die Teilnahme an internationalen

Tab. 1. ¹³⁷Cs- und ¹³⁴Cs-Aktivitäten in den Organen des Rehwildes. ⁺ = 0,0 bedeutet, dass mindestens 50 % der analysierten Proben unter der Nachweisgrenze des jeweiligen Isotops gelegen sind.

Muskulatur	n	$\tilde{x}$	$\bar{x}$	$\pm s$	Min.	Max.
¹³⁷ Cs	163	20.9	26.8	31.6	0.1	312
¹³⁴ Cs	162	10.0	12.6	14.7	0.1	142.5
Leber						
¹³⁷ Cs	120	8.7	12.4	12.9	0.2	87.4
¹³⁴ Cs	120	4.1	5.8	6.1	0.1	41.6
Niere						
¹³⁷ Cs	116	16.7	24.0	29.3	0.9	239.0
¹³⁴ Cs	115	7.5	11.0	13.5	0.3	112.0
Milz						
¹³⁷ Cs	106	8.7	12.0	14.8	0.4	120.0
¹³⁴ Cs	106	3.9	5.9	7.3	0.1	56.2
Schilddrüse						
¹³⁷ Cs	71	0.0 ⁺	7.1	15.0	n.n.	84.0
¹³⁴ Cs	71	0.0	2.6	12.3	n.n.	100.0

Ringversuchen und Testmessungen von Referenzmaterialien, deren Radionuklidgehalte durch Ringversuche garantiert sind, ist auch die Genauigkeit der Messungen gewährleistet.

Bei den Messungen wurden die Aktivitäten mehrere Radionuklide erfasst, wovon vor allem Caesium-137 und -134 sowie Jod-131 von Bedeutung waren. Aufgrund der wegen der kurzen Halbwertszeit rasch abklingenden Jod-131-Kontamination wird im folgenden nur auf die Belastung mit Caesium eingegangen.

ERGEBNISSE

In dieser Arbeit werden vor allem die beim Rehwild erzielten Ergebnisse diskutiert, da von dieser Wildart das umfangreichste Probenmaterial zur Verfügung stand und sich gezeigt hatte, dass die höchsten Belastungswerte bei dieser Spezies festzustellen war (TATARUCH, 1988).

In der nachstehenden Tabelle sind die ¹³⁷Cs- und ¹³⁴Cs-Kontaminationen der bis 31.12.1986 analysierten Proben angeführt (alle Angaben in nCi/kg Frischsubstanz - 1 nCi = 37 Bq).

Aus den in Tab. 1 angeführten Ergebnissen ist ersichtlich, dass die Aktivität von ¹³⁷Cs etwa doppelt so hoch wie die von ¹³⁴Cs war. Dies ist damit zu erklären, dass sich in den nach dem Reaktorunfall freigesetzten Emissionen die Aktivitäten von ¹³⁷Cs wie 2:1 verhielten (ANONYM, 1986).

Die höchste Konzentration von Caesium war in der Mehrzahl der Fälle (81,9 %) in der Muskulatur nachzuweisen. Die entspricht der Verteilung des Kaliums im Organismus, dem das Caesium auch chemisch verwandt ist. Unterschiedliche Caesiumaktivitäten konnten bei der Untersuchung der Muskulatur von verschiedenen Körperpartien ermittelt werden: So zeigten Proben vom Rücken um durchschnittlich 5 % höhere Caesium-Aktivitäten als die Proben vom Oberschenkel desselben Tieres. Im Halsbereich waren nur 80 % der Kontamination des Oberschenkels feststellbar. Muskulatur mit einem höheren Anteil an Sehnen wies niedrigere Caesium-Mengen auf.

Die Nieren, über welche Caesium zum grössten Teil ausgeschieden wird, wiesen die zweithöchsten Aktivitäten auf, in einigen Fällen (18,1 %) sogar höhere als die Muskulatur desselben Tieres.

In Leber und Milz waren deutlich niedrigere Rückstände von Caesium festzustellen. In den Schilddrüsen war die nachzuweisende Caesium-Menge am geringsten, in mehr als 50 % der Proben lag sie unter der Nachweisgrenze.

Zwischen den Aktivitäten von Caesium-137 und Caesium-134 in den einzelnen Organen ergaben sich hochsignifikante ($p < 0,001$) positive Korrelationen ($R = 0,844$), ebenso wie zwischen Caesium-137 und Caesium-134 im selben Organ.

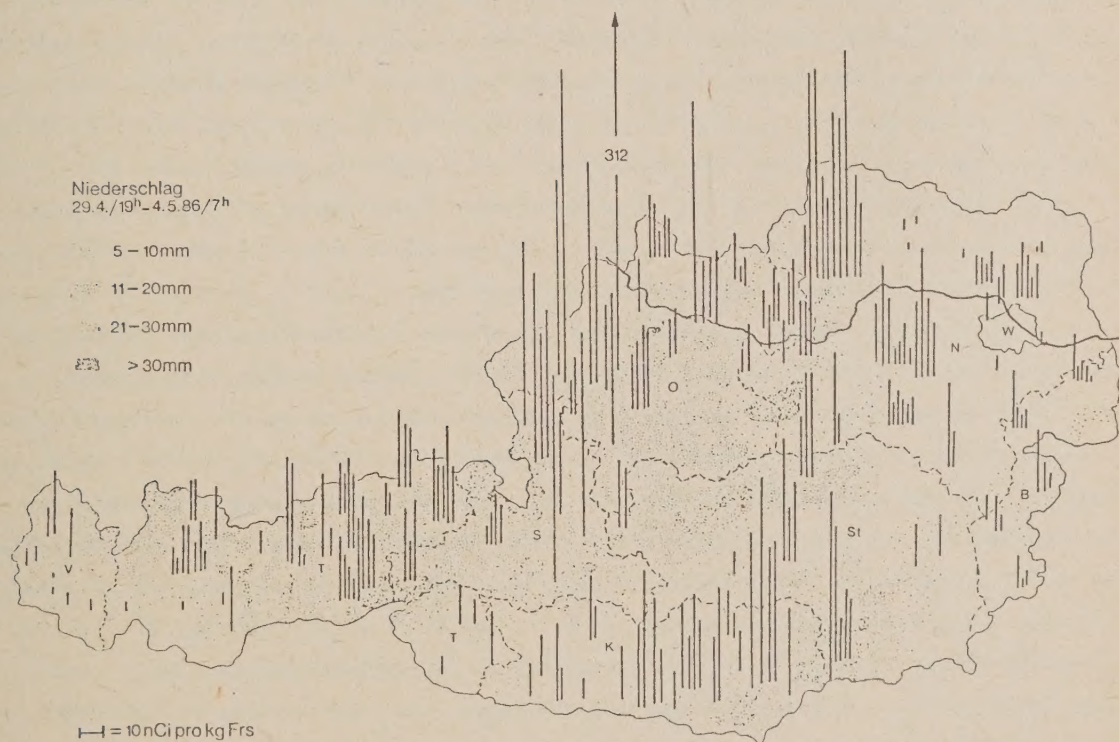


Abb. 1. Regionale Verteilung der ¹³⁷Cs-Belastung der Wildtiere - Vergleich mit den Niederschlagsmengen in der Zeit vom. 29.4./19.00 Uhr bis 4.5.86/7.00 Uhr.

WELCHE FAKTOREN BEEINFLUSSEN DIE HÖHE DER RADIOAKTIVEN KONTAMINATION?

Aus den in Tab. 1 dargestellten Ergebnissen sind die starken Schwankungen der Messwerte erkennbar. Im folgenden soll der Einfluss verschiedener Faktoren, wie Lebensraum, Seehöhe und Erlegungszeit, auf die Höhe der radioaktiven Belastung des Rehwildes diskutiert werden:

Einfluss des Lebensraumes

Für die Untersuchungen des Einflusses des Lebensraumes auf die Belastung der Wildtiere wurden uns auch Analyseergebnisse von acht Landesveterinärdirektionen zur Verfügung gestellt.

Wie aus Abb. 1 ersichtlich ist, waren in den ersten Wochen nach dem Reaktorunfall (es ist der Zeitraum bis 15. Juli dargestellt) sehr deutliche regionale Unterschiede in der Caesium-Belastung des Rehwildes feststellbar.

Diese regional bedingten Differenzen sind auf die stark unterschiedliche Kontamination des Österreichischen Bundesgebietes zurückzuführen.

Die Luftmassen, die durch den Reaktorunfall freigesetzten Radionuklide enthielten, erreichten am 29.4.86 Österreich. Die höchste Konzentration radioaktiver Spaltprodukte in der Luft wurde am 30.4.86 in Wien festgestellt. Sie nahm aber schon in der Nacht zum 1.5.86 deutlich ab. Am 3.5. und am 6. bzw. 7. Mai waren weitere kleinere Anstiege zu verzeichnen. Allgemein war die Radioaktivität der Luft im Osten des Bundesgebietes höher als im Westen (ANONYM, 1986).

Durch die zu dieser Zeit auftretenden Regenfälle, die örtlich sehr unterschiedliche Intensität aufwiesen, kam es zu einem Auswaschen der radioaktiven Nuklide aus der Luft und in weiterer Folge zu einer regional stark variierenden Kontamination des Bodens bzw. der Vegetation. Durch die unterschiedliche Niederschlagsaktivität ist im gesamten Bundesgebiet die Luftbelastung nicht mit der Bodenkontamination korreliert. Die höchsten Belastungen des Bodens bzw. der Vegetation wurden in jenen Gebieten ermittelt, in welchen die höchsten Niederschläge zu verzeichnen gewesen waren und zwar vor allem in grossen Teilen von Oberösterreich und Salzburg, im Westen und Süden von Niederösterreich, im steiermärkisch-kärntnerischen Grenzgebiet, im westlichen Teil Kärntens sowie im Osten Tirols (ANONYM, 1986).

Die Kontamination der Wildtiere korreliert deutlich mit den Niederschlagsverhältnissen und der Belastung der Vegetation. Der höchste festgestellte Wert von 312 nCi/kg Frischsubstanz (11544 Bq) ^{137}Cs und 142,5 nCi/kg ^{134}Cs stammt von einem 6 Jahre alten Rehbock, der am 16.6.86 in Oberösterreich (O) als Fallwild aufgefunden wurde. Die am selben Ort befindliche Messstelle des Österreichischen Frühwarnsystems hatte am 1.5.86 mit einer Dosisleistung von 230 $\mu\text{R/h}$ einen der höchsten in Österreich festgestellten Belastungswerte registriert. Bei einer Regheiss (3 Jahre) aus einem ca. 30 km in westlicher Richtung entfernten Waldgebiet wurde zur gleichen Zeit (24.6.86) eine Kontamination von 123 nCi/kg (4551 Bq) ^{137}Cs und 56,9 nCi/kg ^{134}Cs ermittelt, was den zweithöchsten Wert darstellt. Ein im Auftrag der Oberösterreichischen Landesveterinärdirektion untersuchtes Reh, das am 3.6.86 ebenfalls in diesem Gebiet erlegt worden war, zeigte eine Kontamination von 111 nCi/kg ^{137}Cs und 39 nCi/kg ^{134}Cs . Aus diesen Ergebnissen lässt sich ableiten, dass im Raum Oberösterreich die höchste radioaktive Belastung der Wildtiere gegeben war, was auch durch die grosse Zahl hoher Werte aus diesen Regionen unterstrichen wird (Abb. 1).

Die geringsten Belastungswerte bei Rehwild in Oberösterreich wurden mit 8,4 nCi/kg ^{137}Cs und 5,4 nCi/kg ^{134}Cs im nördlichsten Teil festgestellt.

In dem an Oberösterreich angrenzenden Teil des Bundeslandes Salzburg (S) wurde ebenfalls eine hohe Kontamination beim Wild ermittelt. Nördlich der Stadt Salzburg wurden im Juni bei Rehwild Werte von 83 nCi/kg ^{137}Cs bzw. 25 nCi/kg ^{134}Cs und 92,5 nCi/kg ^{137}Cs bzw. 47,1 nCi/kg ^{134}Cs registriert.

Im Bereich der Hohen Tauern im südlichen Salzburg war ebenfalls eine hohe radioaktive Kontamination festzustellen, die hier von allem an Gamswildproben gezeigt werden konnte und zwar zeigte ein in diesem Bereich erlegter Gamsbock eine Aktivität von 125 nCi/kg ^{137}Cs und 58,6 nCi/kg ^{134}Cs in der Muskulatur.

Die niedrigste Belastung ergab sich bei einem Rehbock aus dem westlichen Bereich dieses Bundeslandes mit 3,9 nCi/kg ^{137}Cs und 2,2 nCi/kg ^{134}Cs in der Muskulatur. Dieser Erlegungsort ist ca. 16 km Luftlinie von jenem Ort entfernt, wo der sehr hoch belastete Gamsbock erlegt wurde. Aus diesem Beispiel ist zu erkennen, wie stark unterschiedlich die Kontamination selbst auf geringen Entfernungen war. Im Lebensraum des Gamsbocker war in der für die Belastung ausschlaggebenden Zeit eine wesentlich höhere Niederschlagsmenge registriert worden als in anderen Gebieten.

In Tirol (T) waren die höchsten Belastungen bei Wildtieren im östlichen Teil des Bundeslandes zu registrieren. Ein erlegtes Reh wies z. B. 52,5 nCi/kg ^{137}Cs und 23,7 nCi/kg ^{134}Cs in der Muskulatur auf. Im westlichen Teil von Tirol waren hingegen sehr geringe Aktivitäten zu verzeichnen, so z. B. bei einer Rehgeiss nur 2,6 nCi/kg ^{137}Cs und 0,7 nCi/kg ^{134}Cs .

Auch in Vorarlberg (V) wurde eine allgemein geringe radioaktive Belastung ermittelt. Der Höchstwert lag bei 30,2 nCi/kg ^{137}Cs und 15,4 nCi/kg ^{134}Cs bei einem Rehkitz, das Mitte Juni erlegt worden war. Im allgemeinen lagen die Werte aber deutlich niedriger und bereits im Juni wurden mehrere Muskulaturproben mit ^{137}Cs -Aktivitäten unter 1 nCi/kg analysiert.

Die deutlich geringere Kontamination von Vorarlberg und Westtirol ist auf die niedrigere Radioaktivität der Luft und auf die geringen Niederschlagsmengen ab dem 29.4.86. nachmittags zurückzuführen. Im Rahmen des bereits erwähnten Frühwarnsystems wurde z. B. in Vorarlberg eine maximale Dosisleistung von nur 34 $\mu\text{R/h}$ registriert (ANONYM, 1986).

Die bereits erwähnt, waren die höchsten Caesium-Belastungen der Wildtiere in Oberösterreich zu verzeichnen. In dem an dieses Bundesland angrenzenden Teil Niederösterreichs (N) lag der Höchstwert bei Rehwild bei 114 nCi/kg ^{137}Cs und 57,1 nCi/kg ^{134}Cs . Diese Belastung wurde bei einem 2-jährigen Bock festgestellt, der am 21.5.86 von einem KFZ getötet wurde. Zwei in geringer Entfernung davon erlegte 1-jährige Böcke waren mit 107 nCi/kg ^{137}Cs und 50 nCi/kg ^{134}Cs bzw. 101 nCi/kg ^{137}Cs und 46,9 nCi/kg ^{134}Cs ebenfalls hoch kontaminiert. Auch weitere Wildtiere aus dieser Region zeigten eine Belastung, die deutlich höher war als die im übrigen Niederösterreich ermittelte. Dies ist auf die hier besonders am 30.4.86 und 1.5.86 erhöhte Niederschlagsstätigkeit zurückzuführen.

Allgemein waren in diesem Bundesland zur kritischen Zeit relativ geringe Regenfälle zu verzeichnen gewesen. Lokal kam es durch Gewittertätigkeit zu höheren Niederschlägen, die sich auch in erhöhten radioaktiven Rückständen im Wildbret bemerkbar machten, wie in dem Gebiet westlich von Wien, wo im Wildbret von Rehwild Werte bis 63 nCi/kg ^{137}Cs und 30 nCi/kg ^{134}Cs gemessen werden konnten.

Tab. 2. Einfluss der Seehöhe auf die Caesium-Kontamination der Wildtiere (Angaben in nCi/kg Muskulatur)

Revier	Tierart	Erlegungs- datum	Seehöhe in m	¹³⁷ Cs	¹³⁴ Cs
Wasserberg	Reh	17.6.86	1260	10,4	4,5
Wasserberg	Reh	17.6.86	1800	37,2	17,4
Zillertal	Reh	26.6.86	1100	17,9	8,8
Zillertal	Reh	18.6.86	1600	41,6	18,9
Achenkirch	Reh	30.6.86	930	9,5	4,4
Achenkirch	Reh	29.6.86	1300	13,3	5,8
Wildalpen	Reh	17.6.86	640	19,0	8,0
Wildalpen	Reh	17.6.86	960	45,0	18,2

Auch im südlichen Niederösterreich konnten höhere Belastungen ermittelt werden, so z. B. bei einem Reh 58,0 nCi/kg ¹³⁷Cs und 28,0 nCi/kg ¹³⁴Cs.

Im Osten und Nordosten dieses Bundeslandes hatte es Ende April / Angang Mai nur sehr wenig geregnet. Diese Tatsache spiegelt sich in einer deutlich niedrigeren radioaktiven Belastung der dort lebenden Wildtiere wieder. Auch die aus dem Bundesland Wien (W) untersuchten Tiere waren gering kontaminiert.

Aus der Steiermark (St) stehen uns leider relativ wenige Werte zur Verfügung, aus denen sich aber eine eher niedrige bis mittlere Kontamination ableiten lässt. Die höchsten Aktivitäten waren im südöstlichen Teil, im Grenzgebiet zu Kärnten (K), festzustellen. Hier war es in der Zeit vom 29.4.86 bis 9.5.86 zu einer der höchsten Niederschlagsmengen von Österreich gekommen. Ein Reh aus dem steirischen Teil wies in der Muskulatur 60,0 nCi/kg ¹³⁷Cs und 27,2 nCi/kg ¹³⁴Cs auf, während bei Rotwild von der Kärntner Seite 94,8 nCi/kg ¹³⁷Cs und 46,8 nCi/kg ¹³⁴Cs und bei Gamswild sogar 135 nCi/kg ¹³⁷Cs und 66,2 nCi/kg ¹³⁴Cs nachgewiesen wurden.

In Ostkärnten war die durchschnittliche Belastung des Wildes höher, so wurden bei einem Reh aus diesem Gebiet 98,9 nCi/kg ¹³⁷Cs und 49,9 nCi/kg ¹³⁴Cs ermittelt. Im westlichen Teil Kärntens war die Belastung deutlich geringer.

Im östlichsten Bundesland Österreichs, dem Burgenland (B), war eine durchschnittlich niedrige Kontamination zu verzeichnen. Der Höchstwert stammt mit 33,0 nCi/kg ¹³⁷Cs und 10,0 nCi/kg ¹³⁴Cs von einem Reh aus dem mittleren Teil.

Einfluss der Seehöhe

Innerhalb desselben Untersuchungsgebietes konnte ein deutlicher Einfluss der Seehöhe festgestellt werden und zwar zeigten Tiere, die in grösseren Höhenlagen erlegt worden waren, eine höhere Kontamination als Individuen der gleichen Art, die tiefer unten erlegt worden waren.

In Tab. 2 sind Beispiele für den Einfluss der Seehöhe auf die Kontamination mit ¹³⁷Cs und ¹³⁴Cs angeführt, wobei immer Tiere innerhalb desselben Lebensraumes verglichen werden.

Tab. 3. ¹³⁷Cs-Kontamination des Rehwildes in Abhängigkeit von der Erlegungszeit (in nCi/kg Frischsubstanz)

Mai	n	$\tilde{x}$	$\bar{x}$	$\pm s$	Min.	Max.
Muskulatur	29	25,7	33,7	21,9	1,4	114,0
Niere	26	22,5	26,0	15,6	6,7	80,0
Leber	24	14,3	16,8	9,3	3,9	42,4
Milz	23	12,8	13,6	6,6	1,8	27,5
Juni						
Muskulatur	80	21,9	29,7	37,9	2,6	312,8
Niere	66	16,1	24,6	32,6	3,1	239,0
Leber	67	8,0	12,5	13,8	1,9	87,4
Milz	51	8,7	13,9	18,2	2,0	120,0
Juli						
Muskulatur	28	21,0	24,3	21,9	2,1	107,0
Niere	12	8,9	15,1	18,2	1,6	66,1
Leber	13	4,0	9,0	8,7	2,4	32,6
Milz	15	3,6	9,3	9,0	0,5	33,8

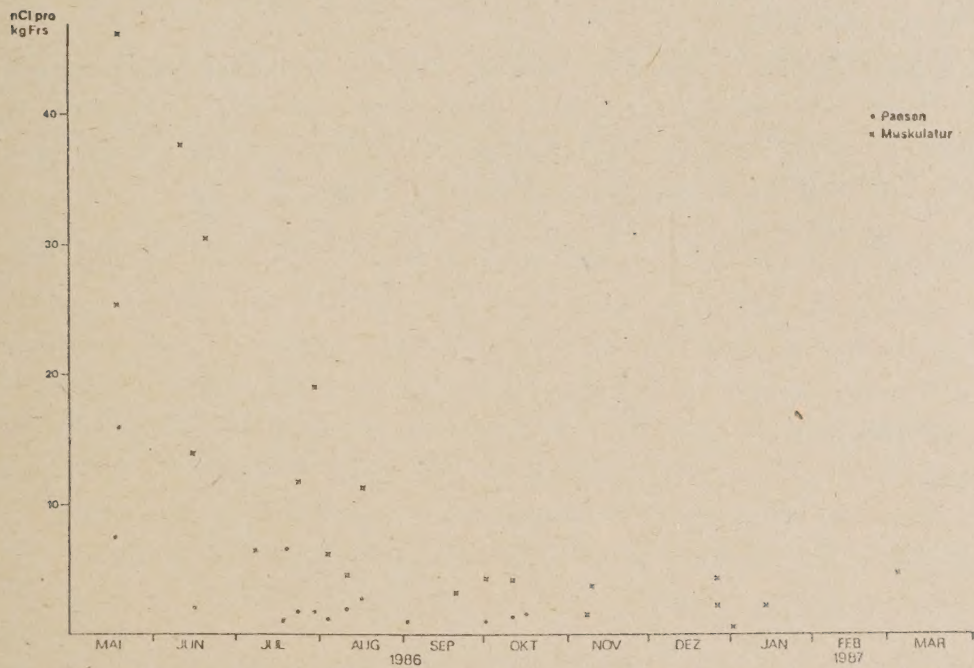


Abb. 2. ¹³⁷Cs-Kontamination von Rehwild (Muskulatur und Panseninhalt) in Abhängigkeit von der Erlegungszeit.

Aus Tab. 2 ist die höhere Belastung der Wildtiere in grösseren Höhenlagen deutlich zu erkennen. Eine der Erklärungen hierfür ist die Tatsache, dass die radioaktiven Luftmassen Österreich in einer Höhe von etwa 750 - 1 500 m überquert hatten (ANONYM, 1986) und überall dort, wo sie auf Berghänge etc. direkt aufprallten, eine höhere Kontamination der dort befindlichen Vegetation verursachten.

Durch Niederschläge kommt es in Berglagen, vor allem oberhalb der Waldgrenze, ebenfalls zu grösseren Belastungen, da hier der Filtereffekt der Waldvegetation nicht zum Tragen kommt. Auch Analysen von Regen- und Schneeproben (ANONYM, 1986) hatten eine Zunahme der Aktivität mit der Seehöhe gezeigt.

Einfluss der Erlegungszeit

Die physikalische Halbwertszeit von ^{137}Cs beträgt 30,2 und die von ^{134}Cs 2,1 Jahre. Daraus ist erklärlich, dass die Caesium-Belastung nicht so rasch abgeklungen ist, wie es beim Jod beobachtet werden konnte, wo bereits im Juni in mehr als 50 % der untersuchten Proben die Aktivität unter die Nachweisgrenze zurückgegangen war (TATARUCH, 1988).

Trotz der grossen physikalischen Halbwertszeit konnte aber bereits im Juli eine merkliche Abnahme der Caesium-Kontamination registriert werden (Tab. 3).

Einen sehr ähnlichen Verlauf zeigt die Abnahme der ^{134}Cs -Kontamination, wo nach einem Medianwert von 12,4 nCi/kg in der Muskulatur im Mai, im Juni noch 10,5 nCi/kg und im Juli 9,2 nCi/kg ^{134}Cs nachweisbar waren.

Dieser Rückgang ist offensichtlich damit zu erklären, dass die biologische Halbwertszeit von Caesium, die von der Verweildauer einer Substanz im Organismus abhängig ist, deutlich kürzer ist. Verschiedene ältere Literaturarbeiten geben sie für Tiere mit etwa 70 Tagen an (BERSIN, 1963). Tiere mit höheren Stoffwechselraten zeigen allgemein auch eine raschere Ausscheidung, so dass für Wildtiere die biologische Halbwertszeit kürzer sein dürfte als für

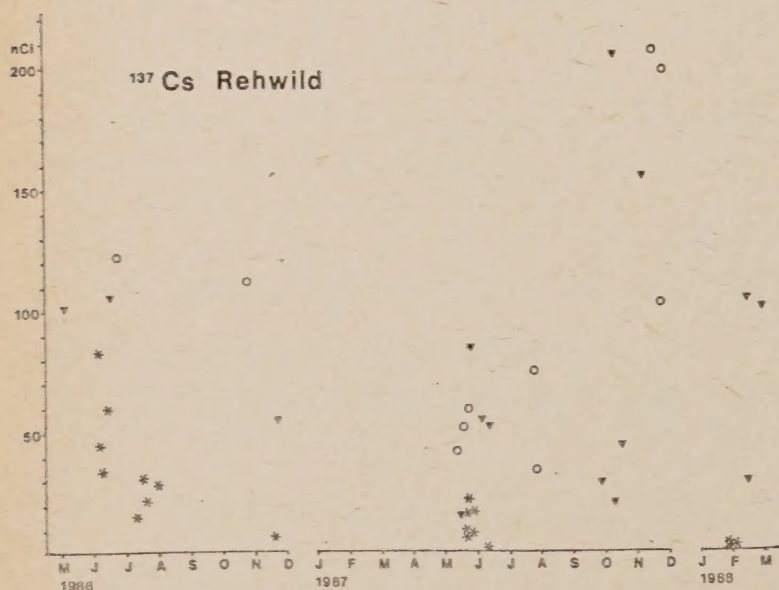


Abb. 3. Zeitlicher Verlauf der Cäsiumbelastung des Rehwildes in 3 Revieren
 ○▽ reine Waldbiotope
 * Wald-Wiesenbiotop etwa 10 km von ▽ entfernt

Haus- oder Nutztiere. Da mit zunehmender zeitlicher Distanz zum 26. April die Kontamination der Nahrung infolge Abwaschung der oberflächlichen radioaktiven Verunreinigungen sowie des Wachstumsfortschrittes etc. geringer wurde, ist auch hiermit der Rückgang der Caesium-Kontamination der Wildtiere zu erklären.

Deutlich ist der Rückgang der radioaktiven Kontamination aus Abb. 2 zu ersehen, die Ergebnisse von Rehwild aus einem in Niederösterreich gelegenen Revier darstellt.

In diesem Revier wurden auch die ^{137}Cs - und ^{134}Cs -Aktivitäten in den Panseninhalten der erlegten Rehe untersucht, wobei bereits zwei aus dem April 1986 miterfasst wurden. Die ^{137}Cs -Belastung in diesen Proben lag erwartungsgemäss unter der Nachweisgrenze. Hier wurden die Analysen bis zum März 1987 fortgeführt und dann beendet, da die Kontamination ein sehr geringes Niveau erreicht hatte.

Die in der Abbildung dargestellte Abnahme der Cs-Belastung des Rehwildes ist für nahezu das gesamte österreichische Bundesgebiet als repräsentativ anzusehen: Die während des Winters 1986/87 fortgeführten Messungen an Wildtieren zeigten einen kontinuierlichen Abfall der radioaktiven Belastung des Rehwildes, sodass ab April 1987 die Analysen nur mehr in wenigen ursprünglich stark belasteten Revieren weitergeführt wurden.

Während in den meisten dieser Gebiete im Laufe des Jahres 1987 die Kontamination weiter abnahm, zeigten sich in 2 Revieren überraschende Ergebnisse: Nachdem im Mai 1987 für das jeweilige Areal relativ niedrige Cs-Aktivitäten ermittelt worden waren, kam es im Herbst zu einem sehr starken Anstieg der Belastung des dort lebenden Rehwildes, wobei teilweise Werte registriert wurden, die merklich höher lagen als die im Mai/Juni 1986 ermittelten Kontaminationen (Abb. 3).

Bei diesen 2 Gebieten handelt es sich um grosse, geschlossene Waldbiotope, wo dem Wild die Möglichkeit der Nahrungsaufnahme ausserhalb des Waldes fehlt. Ähnliches wurde im Herbst 1987 auch in einigen Gebieten Bayerns registriert (HECHT, 1987).

Untersuchungen des Cs-Gehalten von bevorzugten Nahrungspflanzen des Rehwildes zeigten, dass manche Pflanzen extrem hohe ^{137}Cs - und ^{134}Cs -Aktivitäten aufwiesen, wie z. B. Farne, Pilze, Heidelbeerblätter u. a. (TATARUCH, in Vorbereitung). Es muss davon ausgegangen werden, dass Caesium in Waldökosystemen ein anderes Verhalten zeigt als auf Ackerbauflächen und hier offensichtlich sehr leicht pflanzenverfügbar ist und von diversen Pflanzen akkumuliert wird.

Detailliertere Untersuchungen, die 1988 von Umweltbundesamt der Republik Österreich in Zusammenarbeit mit der Bundesanstalt für Lebensmitteluntersuchung und -forschung und dem Forschungsinstitut für Wildtierkunde durchgeführt werden, sollen weitere Aufschlüsse liefern.

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The indication of broad patterns of acidification in the United Kingdom using critical load theory

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I N T R O D U C T I O N

Most studies of the effect of acidic deposition in the U. K. have dealt, at their widest, with a particular pollutant or pollutant combination deposited on a specific catchment or ecosystem. More broad-scale attempts to quantify the potential risks to U. K. ecosystems have been hampered in two ways.

Firstly only limited knowledge exists concerning the sensitivity of our ecosystems and their constituent species, and secondly (and perhaps more crucially) information on the concentration and deposition of acidity and other ions over the country was, until recently, fragmentary. However, since the provision of a much wider acid rain monitoring network in 1986 a more complete and detailed picture of acidic inputs is now available (WSL, 1987).

Data on the sensitivity of most ecological systems (both in the U. K. and abroad) is still largely insufficient to allow clear definition of dose-response curves but for some systems quantitative relationships have been obtained from estimates of deposition over time and the observed level of damage. If this knowledge is combined with simplified assumptions concerning chemical equilibria and the buffering capacity of the system, then a critical deposition load for a given pollutant can be calculated.

For the purpose of this study we have adapted the protocols set out in the Nordic Council Critical Load Workshops (NILSSON, 1986, 1988) which quantify critical loads for forest soils, groundwaters, surface waters and nitrogen deposition where the critical load, defined by UNECE Working Group on Nitrogen Oxides (February 1988), is:

"A quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to our present knowledge".

M E T H O D S

In applying the Nordic critical concept to the U. K. three major determinants have been taken into account - pollutant distribution, patterns of land use and the distribution of acid soils and lithologies.

The spatial distribution of acidity and relevant ions are described from two major sources - 'United Kingdom Acid Rain Monitoring' (WSL, 1987) and 'Acid Deposition in the United Kingdom' (RGAR, 1987). Figures for wet deposition of acidity, non-marine sulphate, nitrate and ammonium are given in the former whilst dry deposition data for sulphur and nitrogen species were derived from models in the latter. Some secondary figures for wet and total ammonium come from the model of ASMAN et JANSSEN (1987).

Geology and soil-type will also determine sensitivity to acidic depositions; using the data from 'Acidity in United Kingdom Freshwaters' (AWRG, 1986) we have defined areas of acid susceptibility where acid soils (Fig. 1.) overlie hard, slow-weathering rocks.

BOHÁČ J., RŮŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deteriorationis Regionis. Institute of Landscape Ecology CAS, České Budějovice, pp. 393-399.

Land-use patterns used for the analysis are based on a system by Lawson et Callaghan (in LAST, 1983) and describe the current distribution of four broad types of vegetation, natural and man made, in Great Britain (Fig. 2.). Individual categories of land use tend to be concentrated in different parts of the country and will thus be exposed to substantially different pollutant regimes, in terms of both wet and dry deposition.

To gain an overall perspective of risk from the complex distributional patterns of the three major factors above, some simplification is necessary. To achieve this the broad geographical trends in pollutant concentrations have firstly been synthesized, creating a 'pollution climate' map (after FOWLER et al., unpubl.) of the U. K. (Fig. 3.).

This was taken as a template upon which the distribution of acid susceptible areas and patterns of land use were imposed to try and identify areas most at risk. Within the categories of the pollution climates we have also identified areas with high and low deposition levels (Tab. 1.). To compensate for this low resolution of scale and strike nearer to the 'nil damage' concept of critical load we have also highlighted specific areas of risk (critical systems) within the pollution climates. It should still be noted however, that potentially sensitive ecosystems could still exist on an extremely localised basis, even within areas (such as Climate 2/3) where pollutant levels are not generally deemed to cause concern.

In our assessment of critical loading we examine in turn the concepts presented by Nilsson et al. as they apply to U. K. forest soils, surface waters and the deposition of nitrogen.



Fig. 1. Distribution of acid soils in the UK. (from AWRG, 1986).



Fig. 3. Pollution climates of the UK. (after Fowler et al.).

Tab. 1. Pollution climate characteristics.

Pollution climate	Characteristics	Synopsis of risk
Climate 2	Rural areas receiving moderate pollution, $\text{SO}_2 + \text{NO}_2$ 7.5 ppb Pollutant input dominated by dry deposition	high depositions, uplands: South Pennines high depositions, lowlands: heathlands
Climate 2/3	Pollutant concentrations on a gradient between Climate 2 and Climate 3 Inputs still dominated by dry deposition	lower risk area concentrations median: majority of areas under agricultural practice - soils well-buffered
Climate 3	Remote rural/upland areas, low concentrations of SO_2 and NO_2 Wet deposition dominates	ion concentrations low: Northern Scotland, most of Wales, West Country ion concentrations high: Trossachs, Galloway, Lake District, Snowdonia

Any examination of groundwaters, the fourth category of systems at risk, is omitted due to the lack of comprehensive data for the U. K.

RESULTS

Forest soils

A critical load of $15 - 20 \text{ keq} \cdot \text{H}^+ \text{ km}^{-2} \text{ yr}^{-1}$ is defined by NILSSON et al.; this load, in the bulk of European soils, should cause no decrease in pH nor mobilize potentially toxic cations (particularly aluminium) in the soil solution. To achieve this situation the acid load must not exceed the weathering rate of silicates.

We have used the U. K. figures for wet deposited acidity (WSL, 1987) as our measure of the hydrogen ion (H^+) input to the proton budget. In doing this we have assumed that the dry



Fig. 2. The distribution of vegetation, natural and man-made (from LAWSON, CALLAGHAN, in LAST, 1983).

deposition of acid compounds and the proton production associated with nitrogen transformations are negligible or balanced. From this assumption, a total deposition of $10 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ is equivalent to an average acidity in wet deposition of between pH 4.7 (500 mm annual rainfall) and pH 5.0 (1000 mm annual rainfall).

Climate 3 - overall assessment: Most areas in this pollution climate will be receiving on average $20 - 30 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ with the upper figure being exceeded in relatively few areas. Large areas of afforestation and natural vegetation come within this zone, notably in the West and North-West of Scotland and parts of North Wales. These are also areas dominated by acid soils with little buffering capacity and it is likely that proton inputs will exceed the weathering rate of base cations. However this would probably occur even under unpolluted conditions due to natural long-term acidification (NILSSON, I., 1986).

The majority of Wales and the South-West of England have better buffered soils, mostly under agricultural grassland (leys and pastures), and the stated loads give little cause for concern.

Worst cases: Climate 3 does contain zones with much smaller areas of acid soil and afforestation which are subjected to much higher deposition rates of wet deposited acidity. These levels could possibly exceed the critical load by up to a factor of four. Deposition rates in excess of $50 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ are recorded for the Loch Lomond (Trossachs area, parts of Galloway, the Lake District, parts of the adjacent North Pennines and Snowdonia (WSL, 1987). Other sources of data for Wales and Scotland provide figures of $60 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ on Plynlimon (collections 1981-5 from RGAR, 1987) and $80 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ on the Cairnsmore of Fleet in 1979 (HARRIMAN et al., 1987). Although there has been a significant country-wide reduction in wet deposited acidity since that time, the levels in all the above mentioned areas give cause for concern.

Climate 2: Despite having a pollutant regime characterised by low precipitation and a predominance of dry deposition, the figures for wet deposited acidity are slightly higher than those quoted for Climate 3. However these levels ranging from $20 - 40 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ are mostly deposited in rural lowlands virtually devoid of acid susceptible areas. 'Poor soils' in these region usually support mixed or deciduous woodland and are relatively base-rich compared to those found under coniferous stands in Northern England and Scotland.

Cause for concern does exist within Climate 2 however, in the uplands of the South Pennines which receive large pollutant loads in the form of wet deposition. A load of $50 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ is exceeded over a broad area (WSL, 1987) with one site receiving annual loads during 1980-82 of over $100 \text{ keq.H}^+\text{km}^{-2}\text{yr.}^{-1}$ corresponding to a pH in rainfall of 4.17 (PRESS et al., 1987).

It should be noted that for all high elevation sites throughout Climates 2 and 3 that the Warren Spring figures (WSL, 1987) do not take into account any change in ionic composition with altitude. In some areas 'occult deposition' could increase the ionic concentration of intercepted 'precipitation' by a factor of two or more (RGAR, 1987), especially to vegetative surfaces (Woodin, LEE, 1987).

Surface waters

Critical loads for surface waters are based on both empirical data and models, relating measured deposition and pH conditions in lakes with low ionic strengths ($20 - 50 \mu\text{eq. Ca} + \text{Mg} \cdot \text{l}^{-1}$).

Assuming that acid loads cause no increase in the weathering of base cations then the alkalinity production of the total catchment should at least equal the effective acid loading. Chemical equilibria will then be maintained, protecting water quality, fish and other aquatic organisms.

The critical load figures have been defined principally in relation to sulphur, as wet-deposited sulphate (SO_4^-). Nilsson et al. acknowledge difficulties in calculating the additional dry deposition component but state that the importance of the dry term will vary between regions. In Norway where most of the critical load figures were derived from the dry input is relatively small, but in areas of Southern Sweden and central and south-eastern England it may exceed the wet component by a factor of three or four (HULTBERG, 1985; RGAR, 1987).

For surface waters a range of loads between $10 - 30 \text{ keq.SO}_4^- \text{ km}^{-2} \text{ yr}^{-1}$ is defined. The lower figure is derived for lakes and streams of extremely low ionic strength ($20 - 40 \mu\text{eq. Ca+Mg l}^{-1}$) and the upper range for waters of greater ionic strength ($50 - 160 \mu\text{eq. Ca+Mg l}^{-1}$). There appear to be almost no lakes or streams in the U. K. with ionic strengths less than $40 \mu\text{eq. Ca+Mg l}^{-1}$, and thus it is the upper figure of $30 \text{ keq.SO}_4^- \text{ km}^{-2} \text{ yr}^{-1}$ which is the most relevant to the U. K. For comparison with U. K. deposition data for wet deposited non-marine sulphate (WSL, 1987) $30 \text{ keq.SO}_4^- \text{ km}^{-2} \text{ yr}^{-1}$ is equivalent to $0.48 \text{ g.S m}^{-2} \text{ yr}^{-1}$.

The U. K. situation: Despite a small decrease in wet deposited sulphate over the U. K. in the last ten years (RGAR, 1987) more than half the country receives more than $0.6 \text{ g.S m}^{-2} \text{ yr}^{-1}$, while there are sizeable areas in North Wales, the Pennines, the Lake District and Southern Scotland which are receiving more than $1.0 \text{ g.S m}^{-2} \text{ yr}^{-1}$. Larger values still can be found at individual sites in Wales on Plynlimon ($1.6 \text{ g.S m}^{-2} \text{ yr}^{-1}$, RGAR, 1987) and the Berwyns ($3.1 \text{ g.S m}^{-2} \text{ yr}^{-1}$, PRESS et al., 1987). The highest figures in the U. K. are from Holme Moss in the Pennines ($4.3 \text{ g.S m}^{-2} \text{ yr}^{-1}$, PRESS et al., 1986) and a site in Galloway ($3.14 \text{ g.S m}^{-2} \text{ yr}^{-1}$, HARRIMAN et al., 1987) although about 60 % of the load from this latter site is derived from marine sources.

A comprehensive appraisal of the waters at risk in this country is hampered by a lack of information, especially on alkalinity. Most records of water quality over the U. K. are in terms of acidity (pH) and these do not form an accurate synoptic picture countrywide nor are they adequate for the detection of long term (ten year or so) trends in acidity (AWRG, 1986).

Although specific areas at risk cannot be accurately identified, it appears that the critical load of $0.48 \text{ g.S m}^{-2} \text{ yr}^{-1}$ for waters with ionic strengths of $50 - 160 \mu\text{eq. Ca+Mg l}^{-1}$ will be exceeded in many parts of the country and will be exceeded by up to a factor of six at individual sites with particularly high rates of deposition. One particular area at risk is Galloway, where surface waters are in the range $52 - 164 \mu\text{eq. Ca+Mg l}^{-1}$ and where deposition rates of $2 - 3 \text{ g.S m}^{-2} \text{ yr}^{-1}$ were recorded in 1979 (HARRIMAN et al., 1987). There is now incontrovertible palaeoecological evidence for acidification in many lakes in this area (BATTARBEE et al., 1985) as would be expected from this analysis of the critical loads.

The deposition of nitrogen

Much more uncertainty exists in applying critical loads for nitrogen to the U. K. Nilsson et al. themselves draw attention to the lack of firm scientific information on the subject and state that the quantitative values provided are, at this stage, tentative. Cautious interpretation should also be made of our own total nitrogen figures, the dry deposition term of which is modelled for acidic species (RGAR, 1987) or estimated for dry deposited ammonium. This estimate that dry ammonium will at least match the wet NH_4^+ component may well be conservative for some areas.

The critical load for nitrogen is defined from a number of ecosystems, from the most sensitive ombrotrophic mires and shallow water communities, to the most tolerant systems such as managed conifer forest stands. Thus there is some difficulty in adopting a consensus figure for the U. K. but we have arbitrarily adopted a figure of $10 - 20 \text{ kg N ha}^{-1}\text{yr}^{-1}$, quoted for most forest systems.

Climate 3: Over the majority of this area the values for total deposited nitrogen (N) do not exceed or much exceed the critical load of $20 \text{ kg N ha}^{-1}\text{yr}^{-1}$. Values are smallest in the West and North-West of Scotland where totals cannot be envisaged to exceed $10 \text{ kg N ha}^{-1}\text{yr}^{-1}$. This area includes the majority of poor soils and coniferous afforestations. The risk to high elevation oligotrophic waters in this area is also slight, with the deposited load falling short of $15 \text{ kg N ha}^{-1}\text{yr}^{-1}$ - the critical load for surface waters (DICKSON, 1986).

The remaining uplands within Climate 3, consisting of Central and Southern Scotland, the Lake District and parts of Wales will receive higher levels of about $20 - 30 \text{ kg N ha}^{-1}\text{yr}^{-1}$. This level could pose risks to systems with very low productivity (critical load $0 - 5 \text{ kg N ha}^{-1}\text{yr}^{-1}$) but these are unlikely to be found outside of Scandinavia or the extreme north of Scotland.

Climate 2: From our calculations it is within the upland zone of the South Pennines that the largest U. K. nitrogen loads are to be found. A series of estimates based on the figures of the recent Warren Spring report (WSL, 1987), ASMÁN et JANSSEN (1987) and PRESS et al. (1986) would give a range of loads between $25 - 55 \text{ kg N ha}^{-1}\text{yr}^{-1}$. It is not surprising therefore that this area contains the only scientifically documented case of nitrogen effects on a U. K. ecosystem. Here large areas of wetland were originally lost due to acidic depositions combined with airborne metal particulates but it is now the load of atmospheric nitrogen that is preventing ombrotrophic Sphagnum species from re-establishing (PRESS et al., 1986).

Also within Climate 2 there may be some cause for concern in Southern England where levels may be reaching up to $40 \text{ kg N ha}^{-1}\text{yr}^{-1}$. Dutch research on vegetational changes in this type of system has shown experimentally that Calluna/Erica will be replaced by more competitive grasses at levels of $20 \text{ kg N ha}^{-1}\text{yr}^{-1}$ (MEIJER, 1986).

C O N C L U S I O N S

Despite the many stated uncertainties throughout this paper, we would draw some broad conclusions from this study. Overall we would point to surface waters as the systems giving greatest cause for concern over widespread areas. It might also be argued that the best evidence for effects have been found in these systems (BATTARBEE et al., 1988). With the exclusion of

effects on very sensitive mires, the potential risk for the systems within the categories of forest soils and nitrogen deposition is less clearcut. However to turn the argument on its head, there are few areas in the U. K. whose deposition levels fall a long way short of the critical loads.

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Bacterial activity in mountain lakes sensitive to acidification

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Natural lakes in Czechoslovakia are scarce and most of them are situated in the mountains (above 1 000 m a. s. l.). About 90 lakes (medium and small size) of glacier origin can be found in the Czechoslovak part of Tatra Mountains (Czechoslovak-Polish boundary). The Tatra Mountains belong to the Carpathian system, the highest peak reaches 2 655 m, central part is composed of granite, with calcareous inclusions in several valleys. The area is protected as National Park.

Lakes are surrounded by steep slopes, usually with a very thin soil layer and they are both geologically and hydrologically sensitive to acidification. Not many reliable data on chemical indices of acidification are available from the past. However, the absence of zooplankton in some lakes in opposite to previous records suggests the trend of decreasing pH and acid neutralizing capacity (STUHLÍK et al., 1985; FOTT et al., 1987). Comparing chemical composition of waters at the Polish side of the Tatra Mountains (BOMBÓWNA, 1965) with our data, the increased share of nitrates and decreased share of carbonates in the total sum of anions during the last 20 years is apparent (STUHLÍK et al., 1985).

Selected lakes are regularly monitored since 1987 in order to evaluate the long-term trends and to estimate the activity of some biological processes. Among the lakes investigated (Tab. 1) there are:

- one acid humic lake (Ja),
- two lakes with anthropic influence (Št, Po) - introduced fish, hotel at the bank, release of treated sewage in one case (Po),
- three oligotrophic lakes at high altitude (VW, La, VH) with different degree of acidification.

The characteristic features of the lakes are the cyclic yearly variations in chemical composition connected with a sudden input of melted snow and thawing ice in spring and an increasing contact time of water with the bed during summer and autumn (STRAŠKRABOVÁ et al., in press).

In spring, minimum values of pH, conductivity and acid neutralizing capacity were found, which gradually increased towards autumn. The autumnal values in the investigated lakes were: pH 4.4 - 8.2, conductivity 27 - 42 μS , acid neutralizing capacity 2 - 141 $\mu\text{eq.l}^{-1}$, sulphates 4 - 10 mg.l^{-1} , nitrate nitrogen 5 - 650 $\mu\text{g.l}^{-1}$, Ca 1.2 - 6.7 mg.l^{-1} , Na 0.4 - 1.9 mg.l^{-1} , total P 4 - 20 $\mu\text{g.l}^{-1}$, chemical oxygen demand 1 - 18 mg.l^{-1} . Surface temperatures in October reached 4 - 9.5° C without any distinct thermal stratification.

The biotic components also comprised characteristic seasonal pattern (STRAŠKRABOVÁ et al., in press). After the spring input of allochthonous nutrients resulting in bacterial and later phytoplankton peak, a maximum development of total plankton biomass based on the autochthonous primary production occurs towards the autumn. At this period (October 1987), the activity of bacteria and phytoplankton and the biomass of bacterio-, phyto- and zooplankton were measured and evaluated.

Tab. 1. Characteristics of the lakes studied (Ja - Jamské, Št - Štrbské, Po - Popradské, VW - Vyšné Wahlenbergovo, VH - Velké Hincovo, La - Ladové in Velká Studená valley).

	Ja	Št	Po	VW	VH	La
elevation m	1447	1346	1494	2145	1946	2057
surface km ²	0.007	0.198	0.069	0.052	0.201	0.017
maximum depth m	4.2	20	17.6	20	54	17.8
pH range in July-October	4.0 - 4.4	7.5 - 8.4	6.9 - 8.2	4.5 - 5.3	7.3 - 7.7	6.4 - 6.9
remark	humic, trees	fish, trees, hotel	fish, dwarf pine, treated sewage	no tree	no tree	no tree

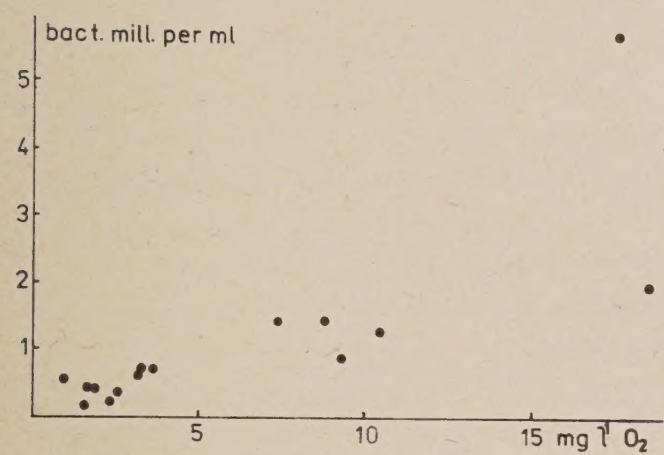


Fig. 1. Bacterial counts in surface layer of the lakes at various concentrations of dissolved organics (COD) in June, July and October.

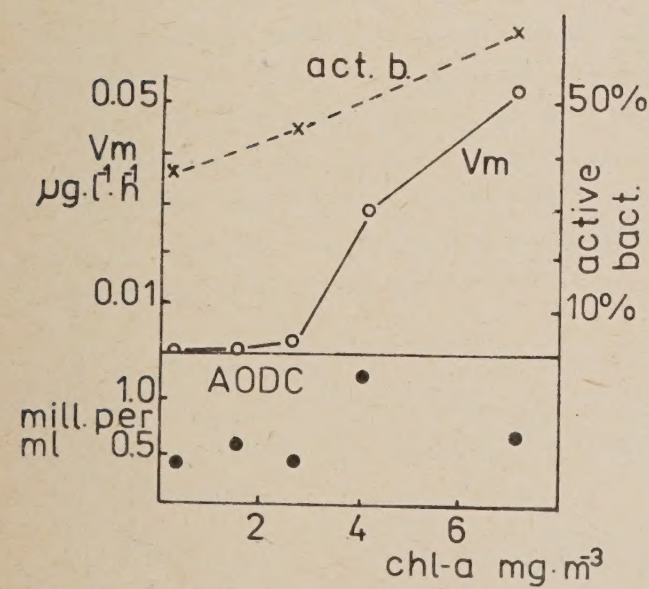


Fig. 2. Bacterial counts (AODC), maximum uptake velocity for glucose (V_m) and percentage of active bacteria (autoradiography- amino-acids) in surface layer of the lakes at various concentrations of chlorophyll-a (in October).

Tab. 2. Estimates of organic carbon in bacteria, phyto- and zooplankton in the lakes and a mesotrophic reservoir Ří, primary production and the chemical characteristics of the surface layer. ND - not determined. Note: The values of primary production in Št lake are low due to bad weather during measurement.

	Ja	Št	Po	VW	VH	La	Ří
plankton org. C mg . m ⁻³							
bacteria	65	42	27	13	14	19	88
phytoplankton	ND	162	280	15	114	61	976
zooplankton	118	106	524	0	53	67	335
total biomass	ND	308	831	28	211	147	1399
% bacteria in tot.	-	14	3	46	7	13	6
primary product. mg . m ⁻² C per h	ND	0.41	49.7	0.21	9.2	8.1	ND
deepest layer measured	ND	12	8	12	25	15	ND
pH	4.5	6.9	7.5	5.3	6.7	6.9	7.1
acid neutr. cap. µeq . l ⁻¹	4.4	141	103	2	135	69	580

METHODS

pH, acid neutralizing capacity and total inorganic carbon concentration was measured in samples from polyethylene bottles using Gran's titration (TALLING, 1973).

Total phosphorus was determined after mineralization with perchloric acid as molybdene blue (POPOVSKÝ, 1970).

Chemical oxygen demand was determined by dichromate oxidation.

Bacterial numbers were counted with epifluorescent microscope after concentrating on membrane filters and staining with acridine orange (ŠIMEK, 1986).

Chlorophyll-a concentration was measured spectrophotometrically after concentration on Whatman GF/C filters, drying and 90 % acetone extraction (LORENZEN, 1967). Zooplankton biomass was determined as dry weight.

Bacterial activity was evaluated : 1. as percentage of metabolically active cells - by micro-autoradiography after incubation with a mixture of tritiated aminoacids (ŠIMEK, 1986). 2. as maximum uptake velocity of glucose - the uptake kinetics at 6 added concentrations of ¹⁴C-glucose was determined according to WRIGHT et HOBBS (1966), detailed description by FUKSA (1985).

Primary production of phytoplankton was measured by ¹⁴C method in five depths, the deepest measured layer corresponded to the double transparency (Secchi disc reading) or near bottom layer if the total column was transparent. After the exposition, samples were fixed by Lugol's solution, immediately filtered through membrane filters and dried. Then the radioactivity was measured by liquid scintillation.

Organic carbon estimates in various components of plankton were performed as follows: 1. from bacterial counts, the total volume biomass was calculated assuming one cell volume of 0.1 µm³, 1 mm³ of volume biomass was assumed to contain 330 mg of carbon (SIMON, AZAM, 1988), 2. phytoplankton fresh weight was calculated from chlorophyll-a by multiplying with 400 (DESORTOVÁ, 1979) and carbon was assumed as 10 % of fresh weight (VINBERG et al., 1971), 3. 50 % of zooplankton dry weight was assumed to be carbon (VINBERG et al., 1971).

THE SHARE OF BACTERIA IN THE TOTAL PLANKTON BIOMASS

In Tab. 2 the organic carbon concentration in the three main components of plankton was shown. The values from a mesotrophic lowland reservoir (Ří - September 1987) are given for comparison. The highest absolute concentrations of bacterial carbon were found in the two lakes with anthropic influence (Po and Št) and in the humic lake (Ja) with allochthonous detritus input. The biomass of phytoplankton also was high in the two lakes with human impact, though it was controlled by a rather high zooplankton biomass (even surpassing the value of mesotrophic reservoir in one case).

The lowest biomasses of bacteria and zooplankton, and the lowest total biomasses as well were observed in the three lakes at high altitude (VH, La, VW). The concentration of total phosphorus in the three lakes was similar, but the phytoplankton and total biomass was significantly different and was positively correlated with acid neutralizing capacity. Thus the lowest phytoplankton, no zooplankton and the lowest total plankton biomass was found in the VW lake, which already has been acidified. This was also the lake with the highest proportion of bacteria in the total biomass. Primary production was very low in the acid lake and, especially, the production per unit of chlorophyll-a, viz. 0.046, was very low compared to 0.36 and 0.16 in La and VH lakes, respectively.

BACTERIAL ACTIVITY

Bacterial numbers are rather correlated with the organic matter (COD) than with chlorophyll-a or pH. In Fig. 1 the positive correlation of AODC counts with COD throughout the year is shown. The allochthonous input of organics with spring ice-thaw and snow-melting and from detritus is important as well as the autochthonous production of phytoplankton. The highest bacterial counts and highest organics concentration were found in the strongly acid humic lake (Ja - see Tab. 2).

Bacterial activity, on the other hand, was closely connected with the chlorophyll-a concentration (Fig. 2). Within the pH range observed, there was no evidence on inhibition of bacterial activity due to acidification as observed in Canadian lakes by RAO et al. (in press). The lowest (almost unmeasurable) values of glucose uptake velocity were found in all the three non-humic lakes (VW, VH, La) at high altitude and lowest temperature - 4.1 to 6° C, irrespective of pH. In the acidified and coldest lake VW the percentage of metabolically active bacteria was similar as in the mesotrophic reservoir at comparable temperatures (STRAŠKRABOVÁ, ŠIMEK, 1984).

C O N C L U S I O N S

In the pH range of 4.1 to 8.2 bacterial counts were not correlated with pH or acid neutralizing capacity. Bacterial counts were positively correlated with organic matter concentration, irrespective of its source (detritus in humic lakes, input during spring thawing, phytoplankton production).

In non-humic lakes at comparable total P concentrations acidification results in the disappearance of zooplankton and in the decrease of phytoplankton biomass, production and photo-

synthetic activity. Consequently, the total plankton biomass was lower and the share of bacterial carbon in the total significantly increased in an acidified lake.

Bacterial activity was found to correlate rather with chlorophyll-a and temperature than with pH or acid neutralizing capacity.

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Influence of low pH (acid rain) on root: shoot relationship and yield of wheat (*T. aestivum* L.)

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INTRODUCTION

Research directed towards an understanding of acidic precipitation effects, low pH of soil and Al^{+++} toxicity on agricultural productivity tested the growth of roots, shoots and yield between cultivars of wheat. Six winter wheat cultivars were tested for tolerance to acidity of soil (with artificially prepared acid rain) and aluminium in laboratory conditions. Experiments took place in greenhouse where the pots were used with three types of soil (different pH, Al^{+++} concentration, artificial acid rain). Influence of three types of soil on root: shoot relationship, volume of roots, dry matter of shoots and yield was tested. On the basis of laboratory experiments some possibilities for prediction of yield and growth of shoot and root were tested.

MATERIAL AND METHODS

Eight winter wheat cultivars (Iris, Regina, Hana, Košútka, Viginta, Sparta, Selekt, Zdar) were tested for tolerance to acidity (pH 4.0-4.5) and aluminium ions. Seven-day-old uniform seedlings were cultivated in three variants of nutrient solution in sand: 1. pH 7, 2. pH 4.5, 3. pH 4.5 with 5.7 mg of Al^{+++} l^{-1} in form of $KAl(SO_4)_2$. Average air temperature in a controlled environment room was 25° C for 16-hr light period and 20° C during darkness. A root tolerance index (RTI) was calculated by dividing dry weight of roots of plants after 14 day growing at pH 4.5 (or with Al^{+++}) by corresponding dry weight in the control variant.

In greenhouse experiments all above named cultivars were cultivated in pots with three types of soil:

- a) clay loamy brown soil with good fertility (till layer) pH 6.5-7.0.
- b) clay loamy brown soil with artificially prepared pH (4.5) and with 4.5 g $KAl(SO_4)_2$ per pot. Acidity of soil was kept by acid (artificial) rain - low concentration of H_2SO_4 .
- c) Podzolic brown soil from the Krušné Mountains, (Bohemia), pH 4.0-4.5.

All three types of soil obtained the same volume of nutrient solution. At every cultivar five replications in every type of soil were cultivated. During vegetation period pH of soil was controlled and volume of roots measured by electrical capacitance (apparatus KAMEK 1 made in Czechoslovakia). After harvest of plants following traits were measured: number of tillers per plant, weight of 1000 grains, harvest index, spike lengths, number of spikelet per spike, grain weight per spike, diameter of blade (under spike), diameter of blade (first internode), sheath length, plant height, flag leaf area, flag leaf volume, sheath of flag leaf + internode under spike length, dry matter of shoots.

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R E S U L T S

Three groups according to sensitivity of cultivars on the basis of root tolerance index were deduced: Iris, Regina, Hana - tolerant cultivars ($RTI=0.90$), Viginta, Sparta - average cultivars ($RTI=0.67$) and Košútka, Selekta, Zdar - sensitive cultivars ($RTI=0.52$). Good possibilities for prediction of yielding traits of cultivars cultivated in above mentioned three types of soil exist on the basis of laboratory experiments (RTI).

If we take results in good soil (with pH 7.0) as a basis 100 % in all three groups, following results are obtained:

The biggest decrease of yield, dry matter of shoot and volume of roots are already in the third group (Košútka, Selekta, Zdar) - that is to say in the most sensitive group. The largest decrease is already in soil with pH 4.5 and Al toxicity. In soil with a low pH and acid rain decrease is a little milder. The lowest decrease in above mentioned traits was in the first group. Yielding potential which is obtained in a good soil in every group of cultivars is not in good relation with decrease of yield, volume of roots, dry matter of shoots in other two types of soil. The lowest yielding potential is in the second group. The best yielding potential is in the first group.

In all three types of groups of cultivars statistically significant correlations between yield, dry matter of shoot and volume of root were already obtained. In case of soil with acid rain all measured morphological traits were bigger than in case of cultivars cultivated in soil with pH 7. Plants are larger but volume of roots is low similarly as a dry matter content in shoots. Root : shoot ratio is unfavourable for yield. The most susceptible morphological traits are flag leaf and second leaf area, flag leaf and second leaf volume, volume of roots and diameter of straw. In case of yielding traits the most susceptible are harvest index, weight of grains and weight of plants. In case of soil with Al^{+++} toxicity large decrease of all measured traits was observed.

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The influence of simulated acid rain on the leaves and on the formation of biomass of some higher plants

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INTRODUCTION

Acid rain is an important factor influencing whole environment. It's importance first came to prominence with the acidification of freshwaters in Scandinavia, Canada and other areas. Acidification of lakes has also been found in Czechoslovakia, for example in Lake Černé and Lake Čertovo (VESELÝ, 1985). In Czechoslovakia the influence of acid rain on forest ecosystems has been studied particularly, and especially the acidification of forest soils (JONÁŠ, 1984). The objective of this work was to investigate the influence of acid rain on several agricultural crop species under greenhouse conditions.

MATERIALS AND METHODS

Simulated acid rain was prepared by mixing 2M H_2SO_4 and 2M HNO_3 in a ratio of 2.8 : 1. The basic solution was then diluted with distil water to pH 2.0 and pH 3.0. The experimental plants were grown under natural light, with temperature in the range 18 - 28° C. Plants were grown in plastic pots (0.19 x 0.19 x 0.25 m); each pot contained either five plants of clover, or one plant sugar beet or then ten wheat plants.

The acid rain was applied by watering the soil and by spraying the plant foliage, either singly or in combination. The volumes used were 500 ml for watering and 1030 ml as aerosol, both being applied twice weekly. The clover was grown for 170 days and the spring wheat for 85 days and the sugar beet for 162 days. The aerosols were applied with a special sprayer.

RESULTS AND DISCUSSION

The plants were sown and grown in pots containing a partly gleyed soil, collected in the outskirts of České Budějovice. The pots were then placed for further growth on a greenhouse bench. For red clover (*Trifolium pratense* L., var. sativum Schr., subvar. praecox Linhard) two cultivars, diploid START and tetraploid KVARTA, were used for comparison. Each cultivar was subjected to three different treatments: The first group with acid spray of pH 2.0, the second was sprayed with pH 3.0 and the third group was the control. All the treatments were additionally watered with tap-water. The plants were harvested after 170 days and the above-ground parts were first air dried and then later overdried at 105° C until constant weight had been achieved. Cultivar START watered with tap-water only (control) had the highest yield, 753 g . m⁻². The next highest yield was the treatment sprayed with pH 3.0 rain; the lowest yield, 299 g . m⁻², was from the group sprayed with pH 2.0 (Tab. 1). In terms of percentage reduction from the control, the acid sprays of pH 3.0 and pH 2.0 lowered yield by 27.8 % and 60.3 %, respectively. However, different results were found for cultivar KVARTA. Control yield was 577 g . m⁻²; application of pH 3.0 spray caused no reduction but

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Tab. 1. Red clover

cultivar	spraying	yield (g d. w. . m ⁻²)
START	0	753
	pH 3	546
	pH 2	299
KVARTA	0	577
	pH 3	602
	pH 2	412

Tab. 2. Sugar beet

acid rain		$\bar{x}$ 1 plant	$\bar{x}$ 1 plant	$\bar{x}$ 1 plant		Sugar content (%)
watering	spraying	fresh (g)	root fresh (g)	above-ground part d. w. (g)	fresh (g)	
water	-	38.0	14.9	2.6	18.0	15.6
water	rain pH 2	44.9	21.9	3.3	23.0	11.6
rain pH 2	-	50.8	29.3	4.1	21.5	15.6
rain pH 2	rain pH 2	29.6	14.1	2.6	15.4	17.3

Tab. 3. Spring Wheat

acid rain		stem d. w.	leaves d. w.	ears d. w.	above - ground plants d. w.
watering	spraying	g . m ⁻²	g . m ⁻²	g . m ⁻²	g . m ⁻²
water	-	376.3	270.5	229.6	876.4
water	water	457.7	204.2	220.0	881.9
rain pH 3	rain pH 3	432.3	232.4	194.9	859.6
rain pH 2	-	494.0	273.2	226.5	113.7
rain pH 2	rain pH 2	525.6	281.2	210.6	1017.4

the strongly acid (pH 2.0) treatment reduced yielded by 29.0 % (412 g . m⁻²). No morphological changes were observed after the application of these acid treatments.

Sugar beet (*Beta vulgaris* L. ssp. *esculenta*, var. *altissima*) cult. Domona was subjected to three different experimental treatments. One group of plants was sprayed and watered with pH 2.0 rain, the second was only watered with pH 2.0 rain and the remaining treatment was the control, watered with tap-water. When the sugar beet was only watered with simulated acid rain, the average yield of whole plant (root + shoot) was enhanced 33.7 % over the average yield of the control plants (50.8 g compared to 38.0 g respectively). Larger differences were found for the roots where in the acid watering treatment root fresh weight was double that of the control.

The acid watering only enhanced shoot fresh weight by about 50 %. Sugar content of both the control and acid treatments was the same, 15.6 %; However the large differences in root biomass meant that beet plants watered with acid rain had larger total sugar contents. Different results were found when acid spray and acid watering were applied in combination. Total biomass was significantly lower (29.6 g from weight), a reduction of some 22.1 % from the control, although yield of sugar was slightly enhanced by 47 % (Tab. 2).

This combined treatment of watering and spraying with acid rain of pH 2.0 therefore caused reductions in the assimilating organ of sugar beet and could be caused by reduction in some metabolic processes, possibly photosynthesis. Reduction of CO_2 assimilation could account for loss in root biomass accumulation. If the leaves are not sprayed and the plant is only watered the acid rain is neutralized in the soil and enhances growth of beet plants. Acid rain contains nutrients (nitrates and sulphates) and is also able to enhance the availability of other nutritive elements in the soil. Neither watering or spraying with acid rain of pH 2.0 produced visible injury on above-ground parts.

Spring wheat (*Triticum aestivum* L.) cult SANDRA was grown in plastic pots in five treatments. The first group was watered and sprayed with simulated acid rain of pH 3.0; the second group was watered with acid rain of pH 2.0 and the third group was sprayed with this acid rain additionally. The fourth and fifth groups were controls, with tap-water being applied as water plus spray, respectively.

No differences were found in shoot dry weight of the controls (fourth group, $816 \text{ g} \cdot \text{m}^{-2}$, and fifth group, $881.9 \text{ g} \cdot \text{m}^{-2}$). When wheat was watered and sprayed with pH 3 rain yield was reduced by 2.5 % although this was not significant ($p < 0.005$). However application of pH 2 rain enhanced yield in both the watering only treatment, shoot dry weight $993.7 \text{ g} \cdot \text{m}^{-2}$ - an increase of 13.4 %; and in the combined treatment where growth was enhanced by 15.4 % to $1017.4 \text{ g} \cdot \text{m}^{-2}$ (Tab. 3).

Application of simulated acid rain of pH 3.0 did not cause any observable morphological changes in the leaves. Acid rain with pH 2.0 did however caused small white and later yellow necrosis (2 mm and smaller). There were found on horizontal parts of leaves where droplets were not lost by run-off.

Thus the above results show that differential sensitivity exists in plants to simulated acid rain applied under greenhouse conditions. The most sensitive was clover followed by sugar beet, with wheat being the least sensitive.

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Roach, *Rutilus rutilus* L., populations as an indicator of lake acidification

M. RASK

I N T R O D U C T I O N

Most bioindicator systems for assessing the degree of acidification of surface waters are based on changes in the composition of benthic invertebrate fauna of running waters along the pH gradient (ENGBLOM, LINGDELL, 1984; RADDUM, FJELLHEIM, 1984). Because fish species differ in their tolerance to low pH (MUNIZ, 1984; MAGNUSON et al., 1984), There should be suitable indicator organisms also among them.

In general, the knowledge on the ecology and physiology of common fish species is at higher level than that of lower aquatic biota. With regards to acidification, this can help to distinguish the pH-induced fish population responses, direct or indirect, from changes caused by other factors. Several fish population parameters have been used to figure the impacts of acidification: number or biomass of fish per unit effort (ROSSELAND et al., 1980), age distribution (HARVEY, 1985), growth (RASK, RAITANIEMI, 1988), and condition of fish (SCHINDLER et al., 1985). In some studies population densities and biomasses have been estimated, either by electrofishing (ANDERSSON, ANDERSSON, 1984) or by marking and recapturing (LAPPALAINEN et al., 1988). Catch statistics have been useful in some cases in evaluating the decrease of fish populations due to acidification (WATT et al., 1983).

This article deals with roach, *Rutilus rutilus* L., as an indicator species of acidification for small lakes in southern Finland and it is based on a test fishing programme carried out in 1985 - 1987 as a part of the Finnish Research Project on Acidification (HAPRO). The main purpose was to analyze the predictability of acid related water properties for different parameters of roach catches. In addition, an attempt was made to evaluate the usefulness of information from local inhabitants.

M A T E R I A L A N D M E T H O D S

Test fishing in 81 lakes (1 to 5 fishings per lake) was performed during summers 1985 - 1987 with a series of 1.8 x 30 m gill nets having mesh sizes of 12, 15, 20, 25, 30, 35, 45 and 60 mm. Total weight of roach in each catch was measured and the total lengths of individual fish were measured to nearest cm for length frequency distributions. For further examination, a random sample of 50 roach per catch was taken, when possible, for age determination and for back calculations of growth (scales, the procedure of Fraser and Lee, TESCH, 1971). Samples for water analyses were taken from surface water of the lakes during the test fishing.

In examining the relationships between roach measures and abiotic factors (correlation analyses, analysis of variance), 4 quantitative and 4 qualitative properties of roach catches were included. The quantitative measures, 2 absolute and 2 relative, were the total number and weight of roach per gill net series, the proportion of roach from the total weight of the catches

and the roach perch relation (weight). The qualitative measures of roach catches were the mean weight, mean length and growth of roach. The latter was presented as length at age 2 and length at age 5 years values.

The abiotic factors included were surface area of the lakes (ha), their altitude above sea level (m) and following water properties: pH, alkalinity (mmol/l), conductivity ($\mu\text{S}/\text{cm}$), colour (mg Pt/l), calcium hardness (mmol/l) and aluminium. The proportions of total and labile aluminium were determined according to LAZERTE (1984).

Local land owners and fishermen were interviewed on the changes in fish status of their lakes. Attention was paid to disappearance of fish species and to clear quantitative changes among fish species.

RESULTS

Roach were present in the gill net catches from 32 of the 81 lakes in our programme, at pH levels 5.1 - 7.6. Alkalinity of roach lakes varied between 0.01 and 0.37 mmol/l, conductivity

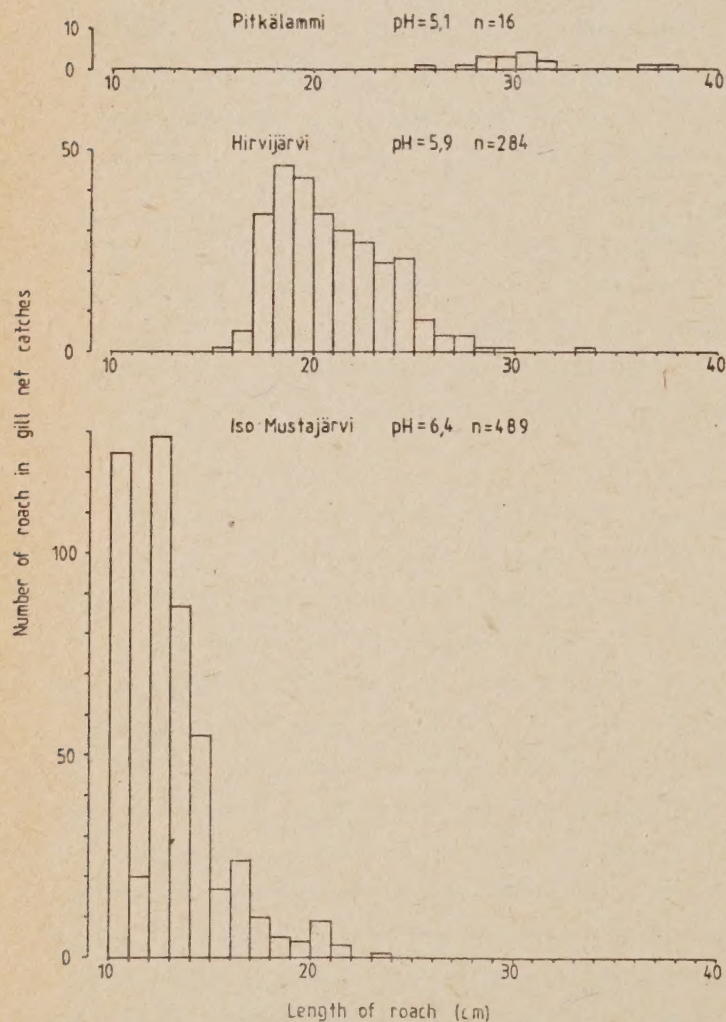


Fig. 1. Length frequency distributions of roach from three lakes with different acidity. The corresponding mean ages of the fish in the catches were 14, 7 and 5 years.

13 - 53 $\mu\text{S}/\text{cm}$, colour 5 - 90 mg Pt/l, Ca-hardness 0.02 - 0.15 mmol/l and total and labile aluminium 0 - 350 and 0 - 190 $\mu\text{g}/\text{l}$, respectively. The surface area of roach lakes varied between 2 and 194 hectares and their altitude was 16 - 154 m above sea level.

At pH levels 5.1 to 5.5 roach were present in 4 of 23 lakes. In the lakes with a higher pH roach occurred more frequently; in lakes with $\text{pH} > 6.5$ in every lake (Tab. 1). Total number and total weight of roach per experimental gill net series were largest at pH levels 6.1 - 6.5 (Tab. 2) as was the proportion of roach from total weights of the catches. Mean length (Fig. 1), mean weight and growth of roach were higher in acid than in neutral lakes (Tab. 2). Also the mean ages were highest in acid lakes, being 7 to 14 years in lakes of pH 5.1 - 5.5 and 3 to 9 years in other lakes.

All the roach parameters considered, except the total weight of roach per experimental gill net series, correlated significantly ($p < 0.05$) with some of the abiotic parameters. According to the number of significant correlations, pH was the best univariate predictor of the roach characteristics (Tab. 3, Fig. 2) whereas altitude and water colour were the worst. In addition to clearly acidity related water properties (pH and aluminium) also properties dealing more with the trophic status of the lakes (conductivity, Ca-hardness) were important (Fig. 2, Tab. 3) as was the surface area of the lakes. The characteristics in roach catches, which differed significantly (ANOVA) among the lake groups with different pH, were the mean length, mean weight and growth of roach (Tab. 4).

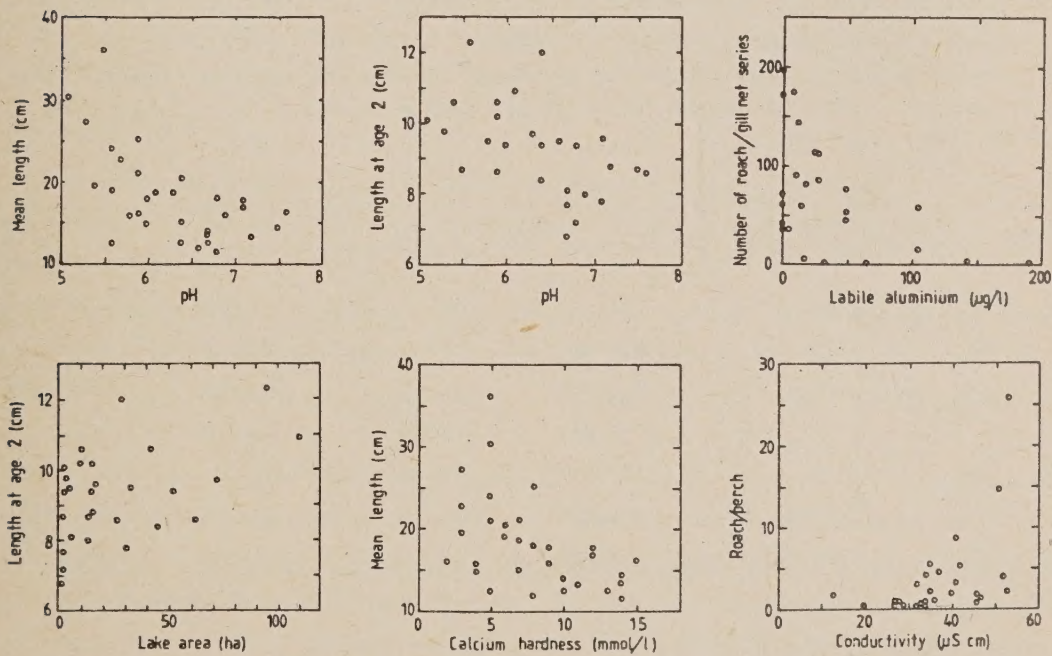


Fig. 2. Examples of the relations between roach catch characteristics and acid related water properties (pH, Labile AL). Lake area, Ca-hardness and conductivity are included to emphasize the possible importance of factors other than acidity on roach.

Tab. 1. Occurrence of roach in lakes along the pH gradient.

pH - range	n of lakes	n of roach lakes	% of roach lakes
≤ 5.0	18	0	0
5.1 - 5.5	23	4	17.4
5.6 - 6.0	18	10	55.5
6.1 - 6.5	10	6	60.0
6.6 - 7.0	7	7	100.0
> 7.0	5	5	100.0

Tab. 2. Mean values of the roach characteristics in 32 lakes grouped according to their pH.

	5.1 - 5.5	5.6 - 6.0	6.1 - 6.5	6.6 - 7.0	> 7.0
N of roach	20 ± 12	48 ± 13	180 ± 67	154 ± 39	73 ± 26
W of roach (kg)	3.5 ± 1.0	4.4 ± 1.4	8.8 ± 1.2	4.5 ± 1.4	3.2 ± 0.8
Roach/tot. catch (W)	0.35	0.34	0.64	0.54	0.56
Roach/perch (w)	0.66	1.28	2.88	5.71	6.87
Mean weight (g)	301 ± 100	85 ± 23	69 ± 13	34 ± 8	50 ± 12
Mean length (mm)	284 ± 34	193 ± 61	173 ± 12	140 ± 9	158 ± 8
Length at age 2 (mm)	98 ± 4	102 ± 5	100 ± 5	81 ± 4	87 ± 6
Length at age 5 (mm)	185 ± 8	175 ± 10	179 ± 7	145 ± 7	162 ± 13

Tab. 3. Statistically significant ($p < 0.05$) correlations between the most important abiotic factors considered, and the characteristics of roach catches (● = $p < 0.05$, ●● = $p < 0.01$, ●●● = $p < 0.001$).

	Area	pH	Al _{tot}	Cond.	Ca-hard.
N of roach	ns	ns	-0.45●	ns	ns
W of roach	ns	ns	ns	ns	ns
Roach/tot. catch	ns	0.44●	-0.41●	0.36●	0.46●●
Roach/perch	ns	0.50●●	ns	0.51●●	0.56●●●
Mean weight	ns	-0.50●●	0.50●●	ns	ns
Mean length	ns	-0.61●●●	0.46●●	-0.45●	-0.49●●
Length at age 2	0.47●●	-0.52●●	ns	-0.42●	-0.58●●
Length at age 5	0.40●●	-0.48●●	ns	-0.45●	-0.52●●

Tab. 4. F-statistics (ANOVA) of roach catch characteristics among lakes groups with different pH (5.1 - 5.5, 5.6 - 6.0, 6.1 - 6.5, 6.6 - 7.0, > 7.0).

	F	df	p
Number of roach	2.61	4, 27	ns
Weight of roach	2.49	4, 27	ns
Roach/total catch (w)	2.49	4, 27	ns
Roach/perch (w)	1.73	4, 26	ns
Mean weight of roach	7.98	4, 27	<0.001
Mean length of roach	9.99	4, 27	<0.001
Length at age 2 years	4.65	4, 27	0.01
Length at age 5 years	3.46	4, 23	0.05

According to interviews of local land owners and fishermen, roach have disappeared from some lakes in the surroundings of Helsinki for as long as 30 - 40 years ago. Today some of these lakes have acidified down to pH 4.5 and are inhabited by decreasing perch, *Perca fluviatilis* L., populations only (LAPPALAINEN et al., 1988). In three lakes, from which we caught only one individual of roach, we were informed that the species has been more abundant in earlier years. In some cases we were told that roach is decreasing in lakes with summer pH 6.1 - 6.5. In most such lakes roach were quite abundant in our gill net catches with no sign of disappearance. However, we measured during the snow melt in April pH values of 5.1 and 5.7 in two such lakes. This may be a symptom of an early stage of acidification.

DISCUSSION

To be a suitable indicator, a species has to be widely distributed, common, well known, easily observed or caught and, above all, it should show a clear response along the environmental gradient considered.

Roach is common in whole Finland except for the northeast part of the country, north of the 68th degree of latitude (KOLI, 1984). In southern and central Finland the presence of roach is not restricted by altitude, 400 - 450 m as given by KEMPE (1962) for Sweden, due to low relief of these parts of the country.

Although there were some significant correlations between the acidity related water properties and quantitative measures of roach catches, attention should also be paid to trophic status of the lakes. This is because the trophic status largely determines the strength of roach in interspecific competition with perch and therefore controls its abundance (SVÄRDSON, 1976). Roach tends to dominate over perch in eutrophic conditions and vice versa (LESSMARK, 1983). However, the critical conductivity level for roach, 40 - 50 $\mu\text{S}/\text{cm}$ as given by NYBERG et al. (1986), seems to be too high because in 19 of our 32 roach lakes the conductivity was <40 and in 6 lakes even < 30 $\mu\text{S}/\text{cm}$. Because the abundance of roach in oligotrophic, acid sensitive lakes seems to be regulated by factors not directly related to acidity, the indicator value of quantitative characteristics of roach catches in a fish status survey may be quite low.

Roach is one of the most sensitive fish species to low pH. The critical acidity for successful reproduction is at pH levels of 5.5 (MILBRINK, JOHANSSON 1975, MUNIZ 1984). Thus, in

15 of our 81 lakes with $\text{pH} < 5.0$, acidity was probably the primary reason for the absence of roach. When the reproduction of roach in a lake fails continuously, the structure of the population tends to change towards the dominance by larger and older individuals. Thus, the highest mean weights and mean lengths of roach in our lakes with $\text{pH} 5.1 - 5.5$ are probably a direct consequence of acid-induced reproduction failures. Lower number of roach in the catches of these lakes in comparison to less acidic lakes support the assumption of decreased populations as do the higher growth rates, which are attributed to the decreased intraspecific competition for food (ALMER, 1972). Because 31 of the 32 roach lakes were also inhabited by perch, it is probable that the food competition between roach and perch still continues although the roach populations are decreasing. This might partly explain the observations that the growth responses of roach due to acidity are not as clear as those observed in perch populations of highly acidified lakes (RASK, RAITANIEMI 1988) but the interspecific differences in the feeding biology of the two species have to be kept in mind.

The information from local fishermen and land owners on the disappearance of roach from some lakes for tens of years ago can help to reconstruct the acidification history of the lakes: an earlier presence of an acid-sensitive fish species indicates that the lakes have acidified recently due to anthropogenic causes. This is important in Finland where naturally acidic small lakes, especially humic ones, are very common. A comparison with corresponding interview data from Sweden (ALMER, 1972) indicates that acidification has affected the lakes in both countries for a longer time than suggested according to paleolimnological studies of some of the lakes. The reason for this discrepancy might be that the low spring pH , caused by snow melting, affects the sensitive fishes but not yet the diatom assemblages commonly used in paleolimnological studies.

Although in some cases the information on the decrease of roach in lakes with summer pH 's > 6.0 could be tied with lower spring pH values, one has to be careful because such information may be simply subjective opinions. Unfortunately, statistics of roach catches over on longer period are probably very rare or nonexistent because of the low economical value of the species.

As a conclusion, roach seems to be a suitable indicator species for acidification of surface waters. Especially at pH levels 5.5, where acidity affects directly the reproduction of roach, qualitative catch parameters like mean weight and length of roach are useful. Because roach is common and well known, the presence-absence information from local inhabitants may help in building up the acidification history of lakes.

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Physiological responses of salmonids as indicators of sublethal stress in acidified organic rivers of Atlantic Canada

G. L. LACROIX

INTRODUCTION

Increased acidification of freshwater ecosystems in the Atlantic region of Canada has resulted in serious effects on fish populations in rivers of the province of Nova Scotia on the eastern seaboard (LACROIX, 1987a, 1987b). Fish in these streams are subjected to chronic sublethal effects over long periods, and to episodic increases in acidity which, in the more acidic streams, result in acute responses (LACROIX, 1985; LACROIX, TOWNSEND, 1987). Physiological methods can be valuable tools in environmental toxicology, subtle changes in the physiology often serving as an early warning of adverse environmental contamination. The dose-dependent nature of a number of physiological and biochemical responses in salmonids to short-term and extended sublethal acid stress in controlled laboratory systems has shown that these could probably be used as relatively sensitive indicators of environmental changes once adapted for field use (FROMM, 1980; WOOD, McDONALD, 1982, 1987; GILES et al., 1984; ADAMS et al., 1985; JONES et al., 1987). These responses could also reflect the degree of chronic stress in fish due to a change in the environment, and serve as early warning signals of impending effects at the population level.

One of the objectives of the studies used in this analysis was to investigate sublethal and lethal responses of juvenile Atlantic salmon (Salmo salar) and brook trout (Salvelinus fontinalis) exposed to chronic acid conditions in streams and to acute acidity typical of episodic events. The purpose of this analysis is then to examine if the responses measured can be used in a field situation to reflect the degree of chronic toxic factor(s) involved in these acidified streams. The latter has become necessary because of the confounding effects of increased aqueous aluminum in response to environmental acidification (LEIVESTAD, 1982). The ionoregulatory, osmoregulatory, and hematological parameters measured in these studies have been identified as responses to either acid alone or acid/aluminum stress (LEIVESTAD, 1982; WOOD, McDONALD, 1987). Therefore, determinations of aluminum accumulated in the gills of fish, which constitute the major target organ (KARLSSON-NORRGREN et al., 1986), and an examination of the surficial morphology of gills, where the effects of aluminum can be observed (KARLSSON-NORRGREN et al., 1986; JAGOE et al., 1987; YOUSON, NEVILLE, 1987), were also included. The usefulness of the measured parameters for detection of toxic responses in natural populations inhabiting acidified waters was examined, the next step being an evaluation of their predictive value for the survival of these salmonids in native streams.

METHODS

The data used in this analysis are from three separate field studies conducted in acidified waters of Atlantic Canada. In 1985, an in situ approach was used to assess the toxicological and physiological responses of juvenile Atlantic salmon during the autumnal decrease in pH

BOHÁČ J., RŮŽIČKA V. (eds.), 1989: Proc. Vth Int. Conf. Bioindicators Deteriorationis Regionis. Institute of Landscape Ecology CAS, České Budějovice, pp. 418-428.

in four acidic rivers of different pH in the Medway River system (about 44° 10' N and 64° 40' W at the mouth) in southwest Nova Scotia. In November 1986 and January 1987, juvenile Atlantic salmon and brook trout were collected from their native streams in the Medway River system. Salmon parr were sampled from 5 rivers and brook trout from 3 rivers during these annual periods of low pH. In 1987, the same in situ approach as in 1985 was used to assess the responses of salmon parr and brook trout to the autumnal acidity episode in 2 sections of an acidic tributary of the Medway River system. One section had been treated by the addition of limestone gravel to the streambed to evaluate the method for enhancing salmonid rearing habitat in small acidic streams, whereas the upstream section remained untreated. The results of the 1985 study, where fish were held in floating pens during exposure, have been published (LACROIX and TOWNSEND, 1987), whereas those of the latter two studies are presented here for the first time.

The methodology was similar in all three studies. Water chemistry was analyzed as described in LACROIX et KAN (1986) and LACROIX et TOWNSEND (1987). Exchangeable aluminum was determined using a modified ion-exchange method; this fraction generally represents the inorganic or potentially labile aluminum present. These reports give complete descriptions of water chemistry in the streams studied. The streams, which represent different pH range groups between about pH 4.4 and 6.2, have significant seasonal pH fluctuations (up to 1 unit). Minimum pH levels in

these salmon rivers usually occur after autumn rains and increases in water flow, and often persist throughout winter. The rivers are also characterized by low calcium concentrations (usually < 1 mg/L), and increasingly high concentrations of dissolved organic carbon (5 - 30 mg/L) at low pH levels. Total dissolved aluminum concentrations are also increasingly high (100 - 350 µg/L) at low pH, but nonexchangeable (organic) aluminum is the dominant form (about 90 %) in these brown waters rich in organic acids.

Fish were held, collected, and sampled as described in LACROIX et TOWNSEND (1987), except for the wild fish which were collected as per LACROIX (1985).

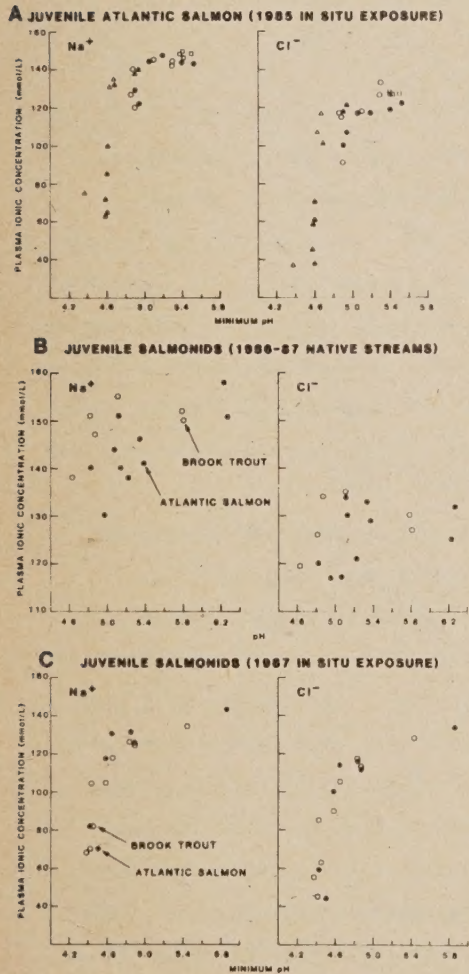


Fig. 1. Effect of water pH on plasma sodium and chloride concentrations in: A. Atlantic salmon parr exposed in separate streams (represented by different symbols) in autumn 1985; B. Atlantic salmon parr and brook trout captured in native streams in autumn 1986 and winter 1987; C. Atlantic salmon parr and brook trout exposed in limestone-treated and untreated areas of a stream in autumn 1987. Each point represents a mean ($N \geq 8$).

Salmon parr and brook trout sampled in these studies were mostly 8 - 10 cm. Blood was sampled, hematocrit was determined, and plasma was analyzed for major ions as per LACROIX et TOWNSEND (1987). Plasma sodium and chloride concentrations are the only electrolytes used in the present analysis because of the lack of consistent relationships between other cations and pH or duration of exposure. Plasma osmolality was also measured in the 1987 study on duplicate 8 - μ L samples with a Wescor 5100 C Vapor Pressure Osmometer. Gills were dissected out and prepared for metal analysis using a dry ashing-acid dissolution method (LACROIX, TOWNSEND 1987). Although both aluminum and iron concentrations in the gills were determined, only aluminum data are presented here because iron content did not vary significantly with duration of exposure, at different pH, or among fish from different streams. Gills were also collected for morphological examination by scanning electron microscopy (SEM). Complete gills were fixed with a mixture of 1.5 % paraformaldehyde and 1.5 % gluteraldehyde in a 0.1 M sodium cacodylate buffer containing 0.05 % calcium chloride. The second holobranch from each gill arch was dissected and postfixed in 1 % osmiumtetroxide, dehydrated through a graded ethanol series, and critical point dried using carbon dioxide. After drying they were mounted on aluminum stubs with silver or carbon paste, and coated with gold in a sputter coater. Gill features were examined at 20 KV in a Cambridge S4-10 stereoscan.

RESULTS AND DISCUSSION

Blood Parameters

There was a distinct relationship between plasma electrolytes of juvenile salmonids ($[Na^+]$ and $[Cl^-]$) and minimum pH recorded in the period preceding blood sampling in all three studies (Fig. 1). This exponential decline in plasma $[Na^+]$ and $[Cl^-]$ with decreasing pH generally translates into an inverse linear relationship with increasing $[H^+]$. The relationship is better defined for fish from the in situ exposures than for fish from native streams. This is probably because of the larger number of samples at lower pH levels (4.4 - 4.8) for in situ experiments, because salmon parr and brook trout were not found in streams with minimum pH < 4.8 and < 4.6 , respectively. However, acclimation to chronic acid stress over the long-term or greater resistance of the wild fish are also possibilities. The overall trend is nevertheless similar, and low $[Na^+]$ and $[Cl^-]$ occurred at the lowest pH levels (Fig. 1B).

Normal plasma $[Na^+] \geq 140$ mmol/L and $[Cl^-] \geq 120$ mmol/L occurred at pH ≥ 5.0 . Conditions were lethal to both species at pH ≤ 4.6 in these studies and plasma $[Na^+]$ and $[Cl^-]$ were < 100 mmol/L and < 80 mmol/L, respectively. These low plasma electrolyte concentrations are indicative of ionoregulatory failure which is linked to the death of fish at low pH (WOOD, McDONALD, 1982, 1987). Sublethal responses which occurred at pH 4.6 - 5.0 resulted in graded decreases in plasma $[Na^+]$ and $[Cl^-]$ with decreasing pH. The duration of exposure also affects the loss of plasma ions (LACROIX, TOWNSEND, 1987), and plasma $[Na^+]$ and $[Cl^-]$ were sometimes higher at pH 4.7 than 4.9 because of the relatively short duration of exposure at the lower pH compared to that at the slightly higher level. Nevertheless, the relationship seems consistent enough for the concentrations of blood ions to be used for predicting lethal and sublethal toxicological effects or the degree of stress in salmonid populations in these acidified

rivers. With regards to relative resistance, brook trout generally survived longer than salmon parr during in situ exposure to low pH (≤ 4.6), and they were also found in more highly acidic streams than the salmon parr.

The loss of ionic regulation and decrease in blood electrolytes in acid stressed fish is reflected directly in the plasma osmotic potential. Plasma osmolality, measured in the 1987 study, decreased with decreasing pH < 4.8 , and the decline was extensive at pH 4.4 - 4.5 (Fig. 2). A plot of the fractional contribution of plasma sodium chloride to the total osmotic pressure ($[Na^+ + Cl^-]/\text{osmolality}$) also showed a decline over the pH range (Fig. 2). Plasma sodium and chloride together account for over 80 % of the osmolality of plasma in salmonids at pH > 4.8 , but as pH decreases to 4.4 the fraction of the osmolality not attributable to sodium chloride increases, indicating an increased presence of some other electrolytes or organic molecules.

A decrease in osmotic potential is generally accompanied by a marked drop in blood volume and increase in the concentration of blood cells. There was an inverse relationship between hematocrit in salmonids and pH, especially in the 1985 and 1987 in situ experiments (Fig. 3).

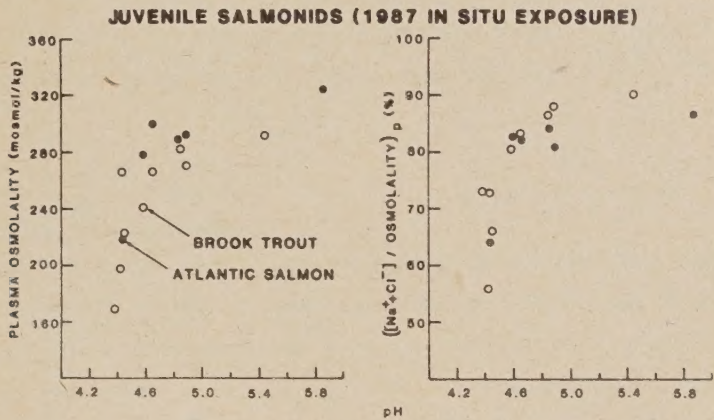


Fig. 2. Effect of water pH on plasma osmolality and on fractional contribution of sodium and chloride to plasma osmolality in Atlantic salmon parr and brook trout exposed in the 1987 study. Each point represents a mean ($N \geq 5$).

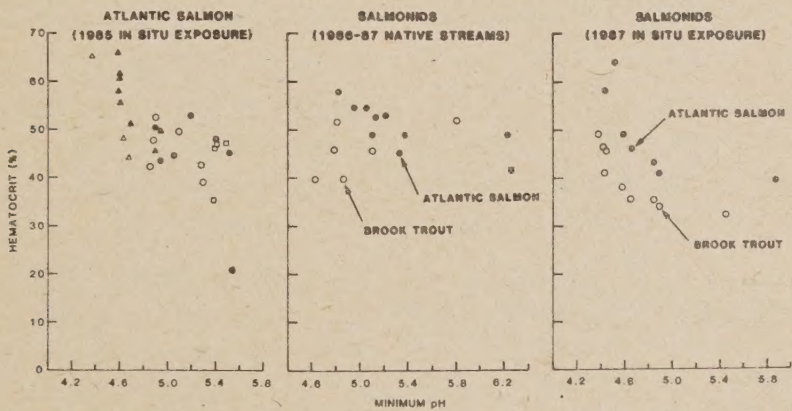


Fig. 3. Effect of water pH on hematocrit in Atlantic salmon parr and brook trout in three separate studies. Each point represents a mean ($N \geq 8$).

The relationship was similar for wild salmon parr in native streams, but not for brook trout. The increase in hematocrit at low pH was also not as large for brook trout than for salmon parr the 1987 study. Normal hematocrit values for salmon parr were about 35 - 45 % and values at sublethal pH levels were as high as 50 - 60 %. Higher hematocrit values were usually indicative of lethal conditions. Hematocrit values for brook trout were consistently lower than those for salmon parr at all pH levels, possibly reflecting a greater resistance to low pH because secondary fluid volume shifts, hemoconcentration, and circulatory collapse are thought to be the ultimate cause of death following ionoregulatory failure in fish (MILLIGAN, WOOD 1982; WOOD, McDONALD, 1982).

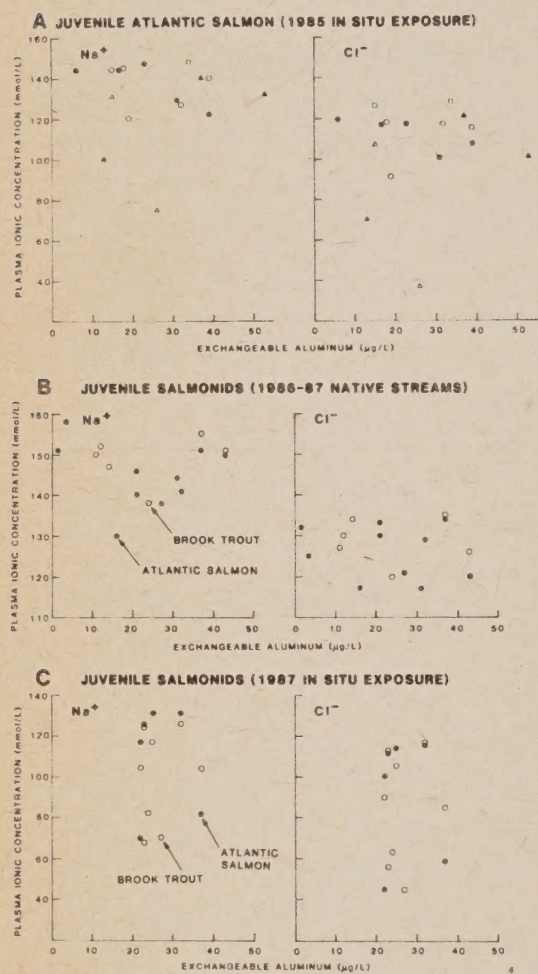


Fig. 4. Plasma sodium and chloride concentrations in Atlantic salmon parr and brook trout in three separate studies in relation to exchangeable aluminium concentration in water. A, B, and C as per legend in Fig. 1. Each point represents a mean ($N \geq 8$).

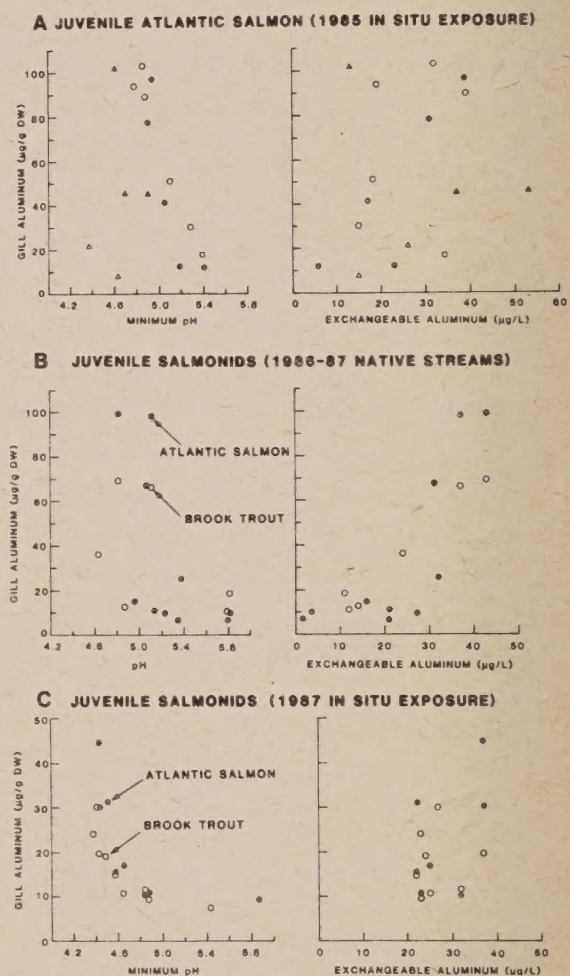


Fig. 5. Concentration of aluminum in gills of Atlantic salmon parr and brook trout in three separate studies in relation to water pH and exchangeable aluminum concentration in water. A, B, and C as per legend in Fig. 1. DW, dry weight. Each point represents a mean ($N \geq 8$).

The possibility of a relationship between the plasma electrolyte loss and other causative factors such as aluminum was also examined. Aluminum toxicity at low pH is generally considered to result in physiological changes similar to those connected with acid alone such as severe ionoregulatory toxicity, but depending upon pH will also result in mild to severe respiratory effects with resultant hypoxemia and acidosis (MUNIZ, LEIVESTAD 1980; NEVILLE, 1985; WOOD, McDONALD, 1987). These changes are mainly a function of the inorganic species or ionic forms of dissolved aluminum (FIVELSTAD, LEIVESTAD, 1984; LEIVESTAD et al., 1987; RAMAMOORTHY, 1988). In all three studies, there was no apparent correlation of plasma $[Na^+]$ and $[Cl^-]$ with exchangeable $[Al]$ in the streams (Fig. 4). Plasma electrolyte concentrations indicative of ionoregulatory failure occurred at exchangeable $[Al]$ as low and sometimes lower than those at which normal plasma $[Na^+]$ and $[Cl^-]$ were measured; the only difference was in pH. Exchangeable $[Al]$ was less than 50 $\mu g/L$ regardless of total dissolved $[Al]$ because of the high organic content of the water in these streams and the direct correlation of total and nonexchangeable $[Al]$ with dissolved organic carbon concentration (LACROIX, KAN, 1986). In streams and at sites with $pH \leq 4.6$, both salmon parr and brook trout died at exchangeable $[Al]$ of 10 - 40 $\mu g/L$, but not at similar $[Al]$ in areas with $pH \geq 4.7$. In contrast, LEIVESTAD et al. (1987) found that the dose/response for Atlantic salmon and aluminum was well correlated with the ion exchangeable fraction.

Gill Aluminum Accumulation

The gill $[Al]$ of salmon parr and brook trout generally increased with decreasing pH or with increasing exchangeable $[Al]$ depending upon the stream or study (Fig. 5). In the 1985 and 1987 studies, there was a better relationship with pH than with exchangeable $[Al]$, this was similar for both species, and was better in some rivers than in others. For example, there was no relationship in the river with the lowest pH where salmon parr died before any significant accumulation of aluminum in the gills (Fig. 5A). In contrast to fish exposed in situ, salmonids in their native streams had gill $[Al]$ which increased exponentially with increasing exchangeable $[Al]$, but showed a poorer relationship with decreasing pH of the streams (Fig. 5B).

Several studies have shown that aluminum accumulation in the gills of salmonids generally increases in a time- and concentration-dependent fashion (KARLSSON-NORRGREN et al., 1986; WOOD, McDonald, 1987). Aluminum accumulated with duration of exposure in the 1985 and 1987 studies, and any relationship with pH may be purely coincidental because pH also changed (decreased) with time. This accumulation could be time-dependent as a new equilibrium is reached in the gills after fish have been transferred from hatcheries to stream cages. Gill $[Al]$ tends to level off if the duration of exposure is long enough (> 30 d) for a new equilibrium to be reached, and it is then independent of the pH trend (LACROIX, TOWNSEND, 1987). The relationship with exchangeable $[Al]$ may be better in fish from wild populations because equilibrium with ambient conditions has probably been reached over the long-term. The extent of aluminum accumulation in gills was considerably less (about 50 %) in the 1987 study than in the 1985 study or in wild fish collected in 1986 - 1987 at similar pH and exchangeable $[Al]$ (Fig. 5), and this may also be related to the shorter duration of exposure in the 1987 study than in the other two studies.

The surficial uptake of aluminum on or within the gill cells of salmonids is generally considered to be a very sensitive indicator of toxicity and well correlated with decreases in plasma electrolytes (NEVILLE, 1985; WOOD, McDONALD, 1987; YOUSON, NEVILLE, 1987; RAMAMOORTHY, 1988). Therefore, the potential for aluminum to act as an accessory factor in the lethal effects observed or to add to the ionoregulatory effects at sublethal levels was examined (Fig. 6). In the 1985 study, there is a slight trend for $[Na^+]$ and $[Cl^-]$ to decrease as gill $[Al]$ increases in salmon parr, and the correlation was even better for both species in the 1987 study. However, in some cases, extremely low plasma ionic concentrations were reached even without any accumulation of aluminum in the gills (Fig. 6A). There was no correlation between plasma electrolytes and gill $[Al]$ of wild salmonids in native streams (Fig. 6B), where the extended duration of exposure to ambient $[Al]$ is such that gill $[Al]$ is in equilibrium with exchangeable $[Al]$ (Fig. 5B).

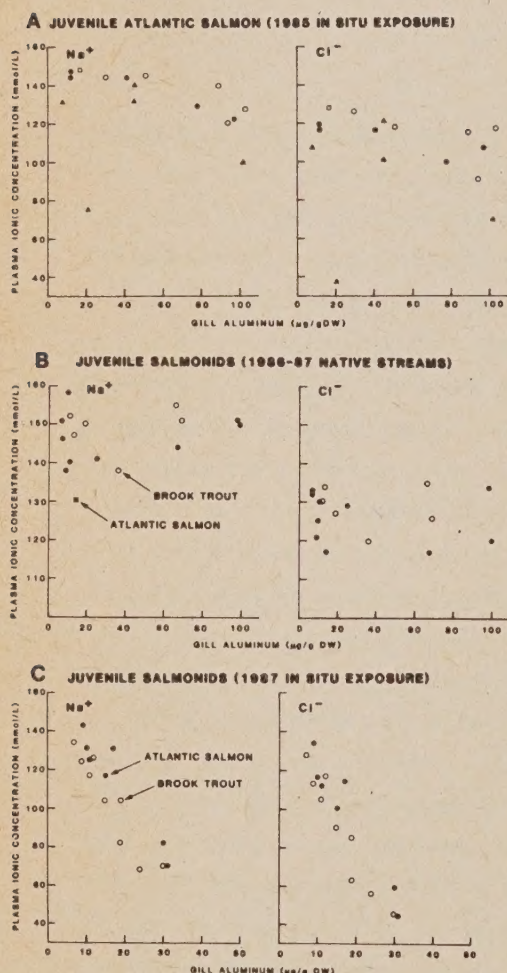
Plasma $[Na^+]$ and $[Cl^-]$ appear to be ultimately linked to the decline in pH rather than to gill $[Al]$, because of the relationship between gill $[Al]$ and water pH and link to duration of exposure as discussed above. The thresholds for decline of these electrolytes and low values occurred at similar low pH levels in the 1985 and 1987 studies (Fig. 1A, 1C), but not at similar gill $[Al]$ (Fig. 6A, 6C). Maximum gill $[Al]$ in the 1987 exposure was less than the concentrations measured before any decrease in plasma electrolytes had occurred in the 1985 study. Accumulation

of aluminum in the gills of salmonids is apparently not the causative factor for the ionoregulatory failure and associated changes responsible for the death of fish at low pH. Conditions were lethal and mortality invariably always occurred at similarly low pH, regardless of the extent of aluminum accumulation in the gills.

Gill Morphology

Morphological examination of gills by SEM showed distinct primary and secondary lamellae with no signs of fusion or hyperplasia for both salmon parr and brook trout from native streams at pH 4.9 - 6.5 and pH 4.6 - 6.0, respectively. The exchangeable $[Al]$ range was 1.3 - 43 $\mu g/L$ in the salmon streams and 11 - 43 in the trout streams. There were no differences among

Fig. 6. Plasma sodium and chloride concentrations in relation to concentration of aluminum in gills of Atlantic salmon parr and brook trout in three separate studies. A, B, and C as per legend in Fig. 1. DW, dry weight. Each point represents a mean ($N \geq 8$).



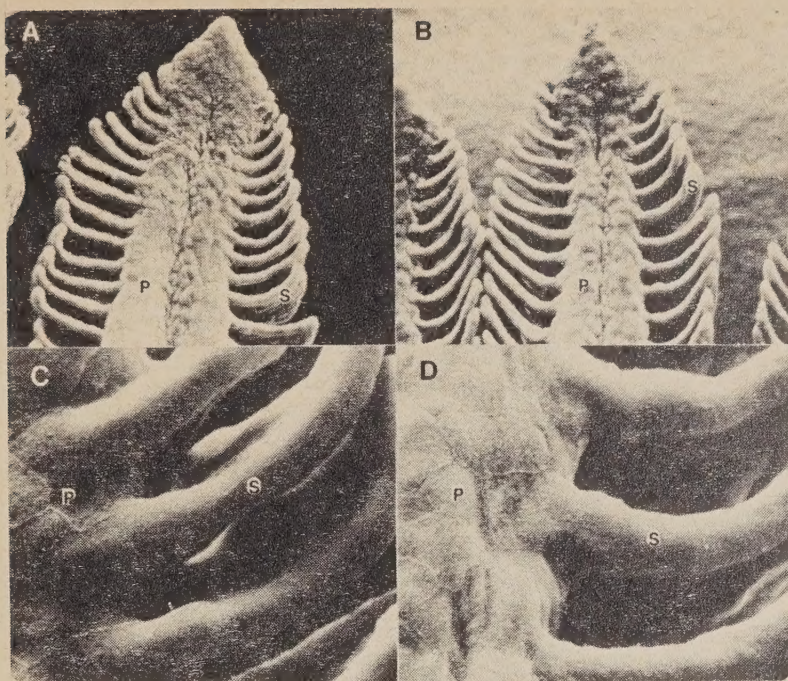


Fig. 7. Scanning electron micrographs of gill filaments of Atlantic salmon from acidic streams showing primary (P) and secondary (S) lamellae. A. November 1986. Magnification, x 240. C. November 1986. x 1200. D. January 1987. x 1250.

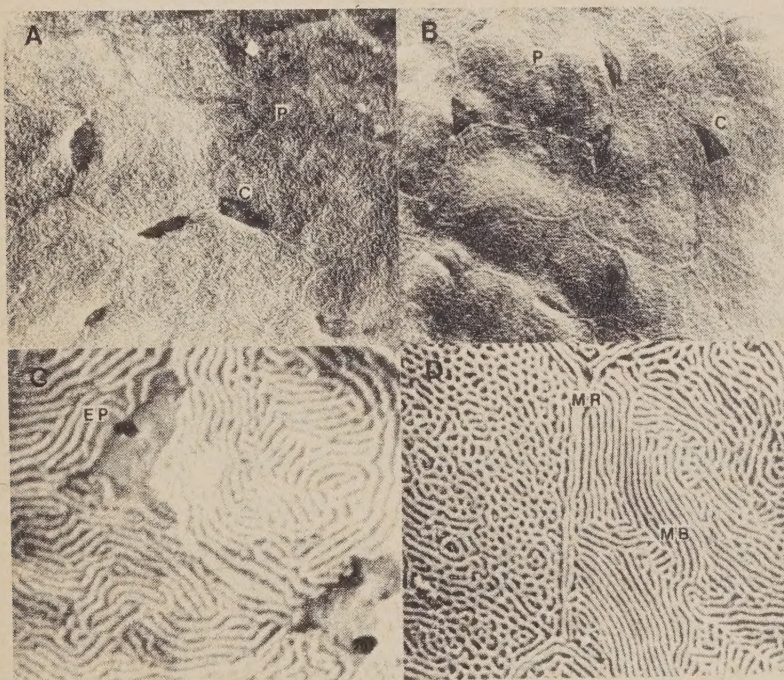


Fig. 8. Scanning electron micrographs of gills of salmonids from acidic streams showing surface of primary lamellae (P), chloride cell crypts (C), epithelial pore (EP), microridges (MR). A. Brook trout, November 1986. Magnification, x 2300. B. Brook trout, January 1987. x 2100. C. Atlantic salmon, January 1987. x 7000. D. Atlantic salmon, November 1986. x 6000.

the gills of fish from streams of different pH or between fall and winter samples. Although there were slightly more mucous droplets on the gills of fish captured in winter, mucous secretion was minimal at both sampling times. These data are from the 1986-87 samples of wild fish and are, therefore, only preliminary results. The general appearance of gill lamellae and epithelium is represented in Fig. 7. The chloride cell crypts were smooth and, in the primary lamellae, were more prevalent proximal to the gill arch (Fig. 8A, 8B). There were no observable differences in the number of crypts at different pH or exchangeable [Al]. Polygonal, epithelial cells or surface pavement cells had distinct thick microridges with thin internal microbridges. Two microridge patterns were observed on primary and secondary lamellae (Fig. 8C), but there were no obvious differences in microridge density for fish from different streams. Epithelial pores, which have been suggested as associated with the chloride cells of the primary lamellae (KARLSSON-NORRGREN et al., 1985), were only observed infrequently (Fig. 8D).

There were, at a range of low pH, none of the gross morphological changes that have been observed in the gills of fish exposed to aluminum at low pH. For example, epithelial cell hyperplasia, fusion between adjacent secondary lamellae, and an increased number of chloride cells bulging out between epithelial cells and causing enlargement of the secondary lamellae have been observed in salmonids under conditions of acid/aluminum stress (KARLSSON-NORRGREN et al., 1986; JAGOE et al., 1987), but not at low pH alone (C. H. JAGOE, personal communication). Exposure to high concentrations of different metals can also lead to intensive mucus production (MUNIZ, LEIVESTAD, 1980), loss of microridges (KARLSSON-NORRGREN et al., 1985), and large non-tissue spaces (TUURALA, SOIVIO, 1982) in the gills of fish. YOUSON et NEVILLE (1987) concluded that increased exposure of chloride cells at the epithelial surface and the accumulation of aluminum in epithelial cells were probably responsible for the toxicity of aluminum to trout in acid environment. Interestingly, KARLSSON-NORRGREN et al. (1986) found that, although the addition of an organic chelating agent such as humus did not affect the accumulation of aluminum at various [Al] and pH 5.5, it reduced or totally inhibited the serious gill lesions which characterized all fish exposed to these conditions without humus. A similar situation would seem to prevail in the organic waters of Nova Scotia, where some aluminum accumulates on the gills of salmonids in acidic streams and there is no evidence of serious effects on the surficial morphology of gill filaments.

C O N C L U S I O N S

The purpose of this analysis was to examine whether physiological parameters could be used to differentiate between lethal and sublethal responses of salmonids to acid exposure, to determine the actual factor(s) eliciting a toxic response, and to indicate the severity of acidification in some salmonid streams of Atlantic Canada. The response thresholds to low pH and correlations in the sublethal and lethal ranges between plasma sodium chloride or osmolality and water pH reflect the sensitivity of these physiological parameters to acid. They can, therefore, be used to predict the severity of acidification in terms of threshold between the zones of tolerance and resistance (sensu FRY et al., 1946). Zones of complete and partial regulation (tolerance), and loss of regulation (resistance) are well delineated by these studies. Complete regulation lies above pH 5.0 for juvenile Atlantic salmon and brook trout. Salmonids exposed to water below this threshold experience a substantial impairment of plasma electrolyte

regulation and osmotic balance, and exhibit hematological abnormalities. After exceeding this pH threshold, the magnitude of the responses were generally proportional to the level of acid stress, and pH levels < 4.7 led to severe ionoregulatory toxicity and loss of regulation leading to death.

The accumulation of aluminum in the gills of salmonids in the Nova Scotia streams did not raise the pH or plasma electrolyte thresholds for loss of regulation and death. The relationship between plasma electrolytes and gill [Al] was apparently coincidental, and there was no consistent dose/response relationship among studies for gill [Al] as there was for pH. Under exposure to low pH plus aluminum, fish generally die with higher plasma electrolytes levels than under acid stress alone because of the respiratory toxicity associated with accumulation of aluminum on or in the gills (WOOD, McDONALD 1987). The increasing importance of these respiratory effects at higher pH in acid/aluminum stress should, therefore, lead to death at higher pH levels than usual. This did not occur in these studies. Measurements of one or more of the parameters indicative of respiratory responses, e. g., blood pH and lactate, and blood gases, would have been useful in separating more definitively that the effects were from low pH rather than from acid/aluminum stress. However, examination of the surficial morphology of gill using scanning electron microscopy revealed none of the cellular disruptions normally associated with aluminum toxicity. Based on the toxicological responses of salmonids and on the dominance of aluminum as nonexchangeable forms in the acidified organic streams of Nova Scotia, LACROIX et KAN (1986) and LACROIX et TOWNSEND (1987) concluded that it was unlikely that aluminum is a lethal factor. The present analysis tends to further support this conclusion.

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Final oration

P. NUORTEVA

The scientists are constructing a common building of knowledge. In order to get a fine and stable building it is important that the scientific workers

- use refined and safe methods,
- tell to each others what they are doing (coordinate the work),
- take care of that the pieces of knowledge are added to each other so that a stable and holistic view of the whole takes shape,
- are critical in order to avoid errors,
- are friendly and collegial in order to do the work on the best way.

International collaboration is necessary to do all this and scientific meetings represent a good instrument for collaboration.

In České Budějovice we have performed all the above normal tasks.

The meeting has been pleasant and important.

Some special dominant features:

1. It exists some economical and political barriers, which inhibits the possibilities of the scientific workers to increase their efficiency by visits in laboratories behind valuta barriers. Solution: We must build up scientific exchange of research workers on institute level or personal level. We must take friendship and collegiality in use. We can not wait the change of political and economic systems.
2. We have developed lots of biomonitors which produce nice description about the situation. This description is, however, often useless for the administrators, because they can not understand the language of the biomonitors. For the scientists too they often say only that some change has happened. The real importance of the biomonitoring is unclear in most cases.

What to do? We must intensify such studies which determines the tolerance limits of the nature and of the public health. The limits must be ecological or ecotoxicological - not purely toxicological. Critical loads are important.

3. Recent monitoring work shows that radioactive cesium from Chernobyl has accumulated into forest humus, detritus feeders and fungi in peak concentrations. This year but not later we have the possibility study the behaviour of cesium in humus ecosystem. People are not aware of this possibility. It is important that we are not missing this unique (?) research possibility.

It is important to alarm all biomonitor colleagues in countries having Chernobyl fallout, to use this opportunity. The conference can do this officially and the members inofficially.

4. We are all happy for the conference, which has well satisfied our waitings, has been scientifically important and inspiring. It has also been pleasant and has risen international friendship and collegiality.

We thank Dr. Boháč and his staff for arranging this conference and the lecturers for their fine contributions.

We hope that the tradition to have these biomonitoring conferences continues in České Budějovice.

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